STUDIES ON HEAVY METAL POLLUTION IN SELECTED EDIBLE BIVALVES OF NORTH KERALA

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DEDICATED TO
MY LOVING PARENTS

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सारांश

उत्तर केरल के नदीमुखों तथा मैंग्रोवों में पाए जाने वाले द्विकपाटी मोलस्क जैसे *क्रासोस्ट्रिया माड्रासेन्सिस, साक्कोस्ट्रिया कुकुलेटा* और *सुनेटा स्क्रिप्टा* में कुछ भारी धातुओं की उपस्थिति और वितरण का अध्ययन करना इस शोध कार्य का प्राथमिक उद्देश्य था. इन धातुओं का स्रोत एवं जमाव की प्रवणता जानने के लिए, जहाँ से द्विकपाटियों को संग्रहित किया गया, वहाँ से अवसाद और पानी भी संग्रहित किए गए. इस प्रकार करने से यह व्यक्त हुआ कि द्विकपाटियाँ पानी की अपेक्षा बार बार भारी धातुओं का जमाव करती हैं. द्विकपाटियों तथा अवसादों में किए गए पूरे अध्ययन काल के दौरान यह मालूम पडा कि सभी स्थानों में नवंबर-दिसंबर महीनों में ज़िन्क का जमाव उच्चतम है और कोप्पर, कैड्मियम तथा मेर्क्युरी का जमाव इससे कम है. पानी के नमूने में कोप्पर ज्यादा पाया गया. द्विकपाटियों में पूरे अध्ययन काल में सभी स्थानों में कैड्मियम की मात्रा अनुमत्य स्तर से अधिक थी और मेर्क्युरी की मात्रा द्विकपाटियों में सभी स्थानों में सिर्फ कुछ महीनों (मुख्यतः अगस्त- सितंबर) में अनुमत्य स्तर से ज्यादा देखा गया. विभिन्न द्विकपाटियों में जमाव की रीति में ज्यादा अंतर नहीं था. भारी धातुओं का जैव आवर्धन *साक्कोस्ट्रिया कुकुलेटा* में सबसे अधिक और इसके बाद *सुनेटा स्क्रिप्टा* में दिखाया पडा. हर स्थान में द्विकपाटियों में भारी धातुओं की माहिक विभिन्नता जानने कि लिए ANOVA का प्रयोग किया गया और कुछ महीनों में देखनेलायक विभिन्नता देखी गयी. विभिन्न नदीमुखों और मैंग्रोव क्षेत्रों से संग्रहित जीवों में किए गए परीक्षण से व्यक्त हो गया कि द्विकपाटियों में होने वाले जलीय अजैव प्राचलों जैसे धातु की सांद्रता और लवणता, तापमान, विलीन ऑक्सिजन और जीव की लंबाई के बीच में कोई प्रत्यक्ष सहसंबंध नहीं देखा गया.

ABSTRACT

The primary purpose of the present work was to study the presence and distribution of certain heavy metals in the bivalve molluscs, *Crassostrea madrasensis, Saccostrea cucullata* and *Sunetta scripta* of the estuarine and mangrove areas of North Kerala. Estimation of metal contents were also done in the sediment and water samples obtained from areas where the bivalves were collected to know the source and accumulation trend. It has been observed that, bivalves accumulated most of the heavy metals several times more than that found in water. Accumulation of zinc was found highest followed by Copper, Cadmium and Mercury in the months of November – December in all stations; during the entire period of study in the bivalves as well as in sediments. Copper was found more in the water samples. While Cadmium was detected above the permissible level in the bivalves in all the stations during the entire period of study, mercury contents were above the permissible limits in bivalves during certain months (mainly August – September) in all the stations. The pattern of accumulation in different bivalves did not exhibit much difference. The biomagnification of heavy metals was maximum in *Saccostrea cucullata* followed by *Crassostrea madrasensis* and *Sunetta scripta*. ANOVA was done for the monthly variation of metal contents in bivalve of each station and observed significant difference in some months. No correlation could be discernible between metal concentration in bivalves and aquatic abiotic parameters such as salinity, temperature, dissolved oxygen and length of the animal collected from different estuaries and mangrove areas.

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INTRODUCTION

1. INTRODUCTION

The tremendous increases in the growth of *human population and the subsequent development* of industries have resulted in the indiscriminate use of many harmful chemicals and pesticides. Their continuous release into the environment has transformed the aquatic ecosystem especially coastal zone into an area, which is highly vulnerable to the deleterious effects of these pollutants. Owing to the harmful effect of pollutants and failure of adequate safety and monitoring measures, these ecosystems are gradually eutrophicated and even some are polluted. Pollution by industrial effluents and domestic sewage especially metals and in particular heavy metals acquire great dimensions due to their prolonged persistence in the receiving environments.

Metals are the constituents of the natural waters. They are essential for the normal life activities and are required at specific levels for the individual organism. While some of these are present in considerably higher levels (sodium, potassium, calcium etc), others like zinc, copper, cobalt are encountered only at trace levels. Essential metals have the potential to be toxic to biota above *certain threshold concentrations. When no biological function could* be attributed or established for a metal, it is usually considered as non-essential. Metals with atomic number of 22 to 92 in all groups from period 3-7 in the periodic tables are included in the heavy metals. Yet another definition is metals of atomic weight higher than that of sodium and having a specific gravity of more than 5 (i.e., densities above $5gm/cm^3$) is termed as heavy metals. Enormous quantities of metals such as arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni), zinc (Zn) *etc.*, have been found their way and continue to be released into the aquatic environment through storm water, waste water and discharge due to various anthropogenic activities and industrial revolution. Many metals both essential such as zinc and copper and non-essential like cadmium, mercury (Zirino and Yamamoto, 1972;

Amiard *et al.*, 1987 and Gerpe *et al.*, 2000), lead, silver and gold are grouped under the category of heavy metals. Most of the heavy metals are enzyme blockers and inhibit especially the enzymes with amino acids, methionine and cysteine.

The dreaded face of pollution of aquatic environment by heavy metals has been exhibited by the great disaster in "Minimata bay", Japan, due to Mercury pollution. Subsequently the gravity of mercury poisoning was encountered in several parts of the world, which resulted in the initiation of detailed research and scientific investigations on the subject. Heavy metals as pollutants of aquatic ecosystem form a major hazard because of their toxicity, persistence and *bioaccumulation/biomagnification in the food chain. It is the soluble compounds of the metals that create the problems* and deleterious effects in the aqueous medium. The electro negativity of metals if more, the toxicity will also be more. Following are the classification of metals based on their toxicity (Mohapatra and Saha, 2000).

1. very toxic effects at concentrations below 1ppm.
2. moderately toxic effects at concentrations between 1 and 100ppm.
3. *scarcely toxic effects rarely appear.*

Sources of some common heavy metals in the water medium:

Zinc: antifouling paints, ship paints etc

Cadmium: mining, metallurgical operation, electroplating industries, sewage, sludge, pesticides, tanning, fertilizer, textile etc.

Copper: mining, metallurgy, algicides, fungicides, paints, corrosion, sewage, fly ash, fertilizer, pulp, paperboard.

Mercury: bulb factories, antifouling paints, pulp and paper mills and pharmaceuticals

Lead: mining, paints, PVC plastics, sewage, fossil fuel, fly ash, exhaust of motor vehicles, oil refineries, fertilizers and steel industries.

Aquatic ecosystems are under the influences of both land areas surrounding them and the atmosphere, which is in close contact with the water at the surface. Among the aquatic ecosystems estuary is a link between marine and freshwater environment as they serve as the usual migratory path, spawning, nursery and feeding grounds for a large number of commercially exploitable and cultivable species. Among the aquatic fauna, almost sedentary molluscan shellfish bivalves like oysters, mussels, clams and scallops are important animals in the ecology of coastal and estuarine waters. Besides effecting substantial improvement in the economy of the fishermen it can also meet the acute protein deficiency of a country like India. Being filter feeders and also bottom dwelling they ingest almost all the materials, dirt and detritus. Hence, their meat is likely to contain large quantity of mud, chlorophyll, sand and microorganisms (Gopakumar, 1988). Apart from this, these organisms have no system in their body by which they can metabolize or destroy the absorbed heavy metals and pesticides thus resulting in bioaccumulation of toxicants. It is also asserted that among molluscs, the bivalves accumulate heavy metals to levels far in excess of those in the hydrosphere (Brooks and Rumbsy, 1965). Eventually it is the human beings who suffer from the results of the presence of toxic metals in the edible aquatic animals. In this context, in the last two decades, biomonitoring programmes have been developed in order to assess the quality of an environment withstanding persistent incoming of pollutants especially of trace metals in coastal and estuarine waters (Phillips, 1977a & Bryan 1976).

In the present investigation, an attempt has been made to evaluate the concentrations of heavy metals like Copper (Cu), Zinc (Zn), Cadmium (Cd), Lead (Pb) and Mercury (Hg) in selected edible bivalves, sediment and water in the estuarine and mangrove environments along the North Kerala in the south west coast of India in order to assess the bioaccumulation in bivalves and the extent of pollution in the environment.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Aquatic animals are exposed to both essential and non-essential metals present in the aquatic environment. Essential ones support biological processes whereas non-essential ones do not have any role. Trace metals taken up by marine invertebrates are accumulated to high body contents and concentrations. Cellular functions are critical to processes involved in metal uptake, regulation, utilization and release. Toxicity can be due to their dysfunction and the resultant interaction of metals with inappropriate cellular structures (Priyalekshmi, 2001).

Perusal of the literature revealed that many studies have been conducted by various authors (Calabrese *et al.*, 1973; Topping, 1973a & 1973b; Boalch *et al.*, 1981; Martin and Flegal, 1975; Denton and Breck, 1981; Balkas *et al.*, 1982; Mathew and Menon, 1983; Frazier and George, 1983; Peerzada and Dickinson, 1988; Peerzada and Dickinson, 1989; Nair *et al.*, 1990; Patel and Anthony, 1991; Bou-Olayan *et al.*, 1995; Senthilnathan and Balasubramanian, 1998; Abreu *et al.*, 2000; Jones *et al.*, 2000; Claisse *et al.*, 2001and Gregory *et al.*, 2002) regarding the influence of heavy metals on the hydrographical features of various aquatic systems and their associated biota.

According to Nambisan and Lakshmanan (1986), several of the trace metals are lethally toxic to fish and shellfish and bioaccumulation of these chemicals will also reduce the quality of fishery products. According to Smith *et al*, (1996) the concentration or body burden of metal in an organism represents the amount accumulated over a period of exposure from the different sources of metals, water, sediment and food.

The effect of the pollutants containing heavy metals has been reported in different aquatic lives by different authors. Jaffer and

Ashraf (1988) reported heavy metals like Cu, Cd, Pb, As etc from the muscle, liver and kidney of long tail tuna (*Thunnus tonggos*) and Indian oil sardine from the coastal waters of Pakistan. High levels of Cu and Zn have been noticed in *Metapenaeus brevicornis* and *Penaeus hardwicki* and in crab *Scylla serrata* from Thana creek. Comparatively higher levels of Copper and Zinc reported from *Charybdis sp.* from Bassein Creek (Ashajyothi and Vijayalekshmi ,1999). Bioaccumulation of mercury by the penaied prawns was asserted by Snehalatha(2000). Concentrations of arsenic, cadmium, chromium, copper, lead, mercury, selenium and zinc were measured in coral, crab, eel, fish, lobster, and sediment samples collected from North Pacific Ocean (Miao *et al.*, 2001). It was found that, crabs (*Grapsus tenuicrustatus*) and undulated moray eels (*Gymnothorax undulates*) exhibited very high levels (3-86 fold) of copper and arsenic, respectively, relative to the other species studied.

Srinivasan and Balasubrahmaniyan (1996) found that the higher concentration of metals in tissues was noted in large size groups and the concentration increased gradually with growth in *Decapterus russelli* (Srinivasan and Balasubrahmaniyan, 1996). Abreu *et al.* (2000) reported the distribution of total mercury (Hg) in *Dicentrarchus labrax* (sea bass) and found that, there is an age effect on the accumulation of mercury in sea bass at the contaminated basin.

Break *et al.* (1976 and 1980) have reported the synergisms and antagonisms between zinc and copper and between zinc and cadmium in marine phytoplankton. Break *et al.* (1976) reported the synergistic effect of copper and zinc on the three marine diatoms and one dinoflagellate except *Phaeodactylum tuicornutum* Bohlin. Bryan (1971) has shown the inhibition of growth of *Laminaria digitata* by zinc, lead and copper. In the ease of zooplankton, Qasim *et al.* (1988) have noted the high concentration of all metals except mercury.

Phillips (1979 a) reported that to a large extent the study of metals in indicator organisms is supplanting that of metals in water or sediments as a means of identifying polluted areas. Studies comparing the relationship between the major differences in geo-chemical characteristics of the sediments and water and the speciation and bioavailability of the pollutant had been carried out by Senthilnathan *et al.*, (1998). They have also studied the differences in the physiological state of the organisms influencing the accumulation and elimination kinetics in the organisms.

Indigenous molluscs are so often used as indicators in pollution studies because of their sedentary and comparatively long life, position in the food web and accessibility (Cairns *et al.*, 1971) and sensitivity to copper, zinc and other associated metals, (Cairns *et al.*, 1976 and Guth *et al.*, 1977). According to Brooks and Rumsby (1965), among molluscs, the bivalves accumulate heavy metals to levels in excess of those in the hydrosphere. Bivalves are known for their capability of acting as efficient time integrated indicators of various metals over a wide range of environmental conditions (Phillips, 1977a; Goldberg, 1975; Phillips, 1978; Phillips, 1980; Pillai and Valsala, 1995). According to Krishnakumar *et al.*, (1990) the suspension feeding bivalve molluscs accumulate and concentrate most of the pollutants within their tissues to concentrations significantly above ambient levels in the environment thus facilitating accurate chemical analysis and assessment.

Oysters, mussels and other bivalve molluscs accumulate a wide range of pollutants from water and food to levels, which are relatively simple to measure. Oysters are extensively used for water quality assessment, as they are potential biological indicator of heavy metal and organic contaminants, similar to the green mussel (Senthilnathan and Balasubramanian, 1998). Theoretically, the study of the content of pollutants in different areas may provide a quantitative estimate of the time integrated ambient contamination of different waste masses (Boyden and

Phillips, 1981). Rajendran and Kurian (1986) reported the positive correlation between the metal concentration in oyster and water and sediment.

Among the heavy metals copper is regarded as a wide spread pollutant in industrialized estuarine areas and is relatively more toxic than other essential trace metals (Sunda *et al.*, 1987, Paulson *et al.*, 1989, Ahsanulla *et al.*, 1981 and Priyalekshmi, 2001). Gerpe *et al.*, (2000) stated that copper is an active center of both the metal enzymes and the oxygen transporting proteins, e.g., hemocyanin super oxide dismutase. According to Talbot and Magee (1978), when present at elevated concentrations, copper and zinc are more lethal to mussels than cadmium. Compared to gastropod, the rate of uptake in oyster was more for Cu and Zn (Krishnakumari and Vijayalakshmi, 1992).

Jones *et al*, (2000) reported that of the many and varied marine organisms chosen as bio-indicators of metal pollution, zinc measurements made on the black lip oyster *Crassotrea echinata* suggested that this organism could be used successfully as a bio-monitoring organism of zinc pollution. It was also reported by him that species differences could cause varying level of zinc in oysters of different species. Gerpe *et al.*, (2000) reported that zinc is a cofactor of several enzymes such as carbonic anhydrase, alkaline phosphatase and DNA and RNA polymerase and inspite of its biological function, both Zn and Cu could produce toxic problems at very high levels.

Greig *et al.*, (1975) suggested that the amount of cadmium and copper transferred from adults of *Crassostrea virginica* to eggs was fairly constant and was not dependent on the amount of metal available in the adult. High level of cadmium accumulation was reported in the hepatopancreas of Mytilus edulis (Theede *et al.*,1979).Cadmium and zinc exert on the activity of the Na^+/H^+ antiporter located on the brush border membranes of eel renal tubular cells (Vilella *et al.*,1999). According to

Waldichuk (1985), certain metals particularly cadmium have been shown to combine with metal binding proteins such as metallothionein in a detoxification process. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax* (Romeo *et al.*, 2000). Talbot and Magee (1976) asserted that oysters accumulate more of cadmium than mussels.

Mercury is regarded as a trace element of concern in marine pollution studies due to its high toxicity. Mercury has been reported to occur at elevate levels in certain 'hot spots' along the Indian coast (Zingde and Desai, 1981).Krishnakumar *et al*, (1987) have studied acute mercury and zinc toxicity in green mussel *Perna viridis*. According to Gregory *et al.* (2002) sub lethal concentrations of mercury were rapidly accumulated in the soft tissues of *Perna perna*, had a negative effect on filtration rates and induced marked changes in gill filament morphology.

There is no evidence for a threshold below which lead has no adverse health effects. Blood lead levels previously considered safe are now known to cause subtle, chronic health effects. According to Mohapatra (1993) no standards have been reported for lead content in different tissues or organs of a fish from which we could evaluate the toxic limits for the same.

Many authors have reported the results of their studies specifically concerning the concentrations of trace metals in water from oceanic areas. Some of these include the pioneering works of Goldberg (1965) for average seawater; Topping (1969) for North Indian Ocean and the Arabian sea; Preston *et al.* (1972) for North east Atlantic; Chester and Stoner (1974) for near shore and open ocean waters of World oceans; Braganca and Sanzgiry (1980) for coastal and offshore regions of Bay of Bengal; Bethoux *et al.* (1990) for Mediterranean sea. The study of heavy metals in the coastal environments in India started during nineteen seventies. Some of the important reports available are the studies conducted in inshore and estuarine waters of the central west coast of India (Sankaranarayanan and Reddy, 1973); in coastal and estuarine waters around Goa (Zingde *et al.*, 1976); in Kodikkarai coastal environments of South east coast of India with seasonal variation of heavy metals (Pragatheeswaran *et al.*, 1988).

Studies by authors Nair *et al.* (1990) on dissolved and particulate metals on overlying water have shown that Cu and Cd concentrations consistently co varied, while Zn, Ni, and Pb co varied on a seasonal basis with other sediment associated metals. The levels of copper concentrated by *Crassostrea cucullata* and *C.gryphoides* in coastal and estuarine waters around Goa have been related to the levels in water (Zingde *et al.*, 1976). The use of sediments to define areas of trace metal pollution has been reported in several studies (Brooks and Rumsby, 1965; Bloxam *et al.*, 1972; Butterworth *et al.*, 1972; Mackay *et al.*, 1972; Talbot *et al.*, 1976). Phillips, (1979) stated that an increase in the trace metal content in the tissue of a benthic bivalve is usually controlled by the increased availability of the trace metal in the bottom sediments. The ultimate sink for elements in solution is the bottom sediment (Hart, 1982). Rajendran and Kurian (1986) reported the positive correlation between the metal concentration in oyster and water and sediment. Bryan (1984), proposed that metals return to solutions by remobilisation from sediment. Livett (1988) asserted that the assessment of marine pollution is not restricted to the study of the water. Most of the pollutants-heavy metals and hydrophobic organic compounds in particular have a tendency to adsorb to sediment particles, and their concentrations in marine sediments may be several orders of magnitude higher than in the water column. According to Wang *et al.* (2002) sediments are considered the sink for metals in aquatic environments because of their strong metal - binding capacity, but they are a potential source for metal ingestion by marine benthic animals as well.

Castanga *et al.* (1982) and Murthy and Veerayya (1981) studied the effect of grain size of sediment and organic matter in the concentration of metals and established a strong correlation between metal value and organic matter content in sediments. They also observed that the elemental concentrations follow closely the texture of the sediment and according to them higher concentration of metal were associated with the silt and clay fraction of the sediment. According to Stevenson (2001), heavy metal concentrations in sediment is known to be influenced by the grain size of the sediment and highest concentration of heavy metals were found in finest fraction of sediment. Mormede and Davies (2001) reported the high concentrations of metals in fine-grained sediments relative to the coarser grained facies.

Regarding the seasonal variation, Rajendran and Kurian (1986) studied the variation of Cu in sediments of Cochin backwater and reported that although there is a monthly variation in metal concentration, there is little evidence of any overall seasonal pattern in sediment. Nair *et al.* (1990) have studied the concentration of a number of metals (Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb and Zn) in sediments of Cochin backwater system before and after flood condition, and have found that there is pronounced seasonal variation in the distribution of trace metals in the sediment coinciding with the flushing of bottom bed material to near shore coastal plains. The variation in the concentration of Zn, Mn, Cu and Fe in the sediments may be attributed to differences in the sources of the heavy metals and complex reactions such as absorption, flocculation, etc. taking place in the sediment (Pragatheeswaran *et al.*, 1986). Athalye and Gokhale, (1989) reported that the lead in sediment exhibited season wise variation with the rise during monsoon and an antagonistic property among the zinc and copper.

Various factors affecting accumulation of heavy metals has been extensively studied in various aquatic organisms by Phillips (1976a and 1976b), Phillips (1977a and 1977b), Wright, (1977) and Vernberg *et* *al.* (1977), Simpson, (1979), Laksmanan & Nambisan, (1983), Unnikrishnan and Balakrishnan, (1986), Mohapatra (1993), Pattersen *et al.* (1997), Senthilnathan and Balasubramanian (1998), Senthilnathan *et al.* (1998) and Jones *et al.* (2000).

Phillips (1976 and 1977a) stated that in the environment, temperature, salinity, and concentrations of other metals present in the water can all influence the uptake of metal by the organism. According to Waldichuk (1985), dissolved oxygen in the water has an effect on metal dissolution in that it controls the redox potential. The effect of heavy metal concentration and the oxygen consumption rate by the organisms has been studied by some authors (Baby and Menon, 1986; Prabhudeva and Menon, 1986; Krishnakumar *et al.*, 1990;Mohanraj and Hameed, 1991; Patel and Anthony, 1991 and Sze and Lee, 2000). Seasonal variation of metals indicating a monsoon maximum and a summer minima has been reported in mussel *P. viridis* and oyster *C. madrasensis* from some parts of south east coast of India (Lakshmanan & Nambisan, 1983, Senthilnathan *et al.*, 1998). A seasonal distribution of metals $\left(Cu^{2+}, Cd^{2+}, \& Zn^{2+}\right)$ with high levels during monsoon season was observed in different organs like gill, mantle and adductor muscle of the oyster *C. madrasensis* has been reported from Uppanar, Vellar & Kaduviar estuaries of south west coast of India (Senthilnathan and Balasubramanian 1998). Where as Mohapatra (1993), noted a very weak negative relation between salinity and copper content in pre-monsoon and post-monsooon. A study conducted by Pattersen *et al.* (1997) has shown that, high concentration of all metals namely Zn, Mn, Fe & Cu were recorded during the rainy monsoon where as concentrations were low during the dry season in edible marine gastropods.

According to Simpson (1979) heavy metal concentration in mussels was found to be related to seasonality of a reproductive cycle. Unnikrishnan and Balakrishnan (1986) stated that one of the major physiological changes, which exert a significant effect on temporal trend in the trace metal levels of higher invertebrates, is the reproductive cycle. Fowler and Oregioni (1976) have also mentioned the relationship between the accumulation of heavy metal and the reproductive cycle of the animal. A study conducted by Muralidharan and Vivekraja(1997) on edible clam *Marcia recens* showed the higher concentration of all the elements (Zn, Cu, Pb) except cadmium in females than in males. They also found that the younger groups concentrate the metals more than the larger size group as observed by Boyden (1977) and Watling and Watling (1976b). Heavy metal accumulation by oysters and other organisms showed species specificity (Zingde *et al.*, 1976; Cuyvers, 1984; Boalch *et al.*, 1981 and Patel and Anthony, 1991; Jones *et al.*, 2000). According to Larsen (1979) copper varied significantly with the age of the organism and cadmium and zinc varied with salinity. The trace metal concentrations within molluscs are reported to depend on size (Romeril, 1971; Raymont, 1972; Boyden, 1974; Phillips, 1976a and 1976b; Boalch *et al.*, 1981). Mackay *et al.* (1975) asserted that metal concentrations decrease with increasing age and wet weight of oysters. In bivalves, effect of age in the uptake of metals was studied by Bryan (1973), Boyden, (1974), and Szefer and Szefer, (1985). Phillips (1977) has also studied the accumulation of metal and size of the animal.

Sundararaj and Krishnamurthy (1972) have well explained their findings on the non-conservative behaviour of heavy metal increase with decrease in salinity. Wright (1977) and Vernberg *et al.* (1977) observed a direct effect of salinity on the rate of uptake of trace metals by marine, estuarine and brackish water organisms. Salinity differences may also strongly alter the bioavailability of pollutants to organisms, and hence may impact toxicity. With cadmium in water, for instance, reducing salinity also reduces chloride complexation, thereby increasing the bioavailability of cadmium to organisms (Mantoura *et al.*, 1978).

Effect of salinity and temperature on the uptake of metal was reported by Phillips (1976 b) and Dougherty (1988). Cunningham (1979) stated that with higher temperature and increased metabolism, more rapid turnover of all tissue constituents including metals would be anticipated. This expectation has been demonstrated by Cunningham and Tripp (1975) using biological half-life as a parameter for comparison. Roberts (1978) stated that the toxicity of heavy metals increase with increase in temperature. According to Phillips (1979 b) a decrease in the trace metal levels in the tissue depends on the biological half life of the metal in the organism the half lives of metals in an organism varies enormously between species and within a species.

MATERIALS AND METHODS

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Selection of sampling stations and samples

Estuaries of Kumbla, Kunjimangalam, Mahe, Kadalundi, Koduvally and Chettua of North Kerala (Plate.1) along the south west coast were selected for the present study. Water, sediment and bivalve samples, *Crassostrea madrasensis,* {(Preston, 1916) (Plate.2)} *Saccostrea cucullata,* {(Born, 1890) (Plate.3)} and *Sunetta scripta,* {(Linnaeus, 1758) Plate.4)} were collected monthly from 6 stations during August 2002 to January 2003. These sites were selected since they may lie adjacent to potential sources of metal pollution.

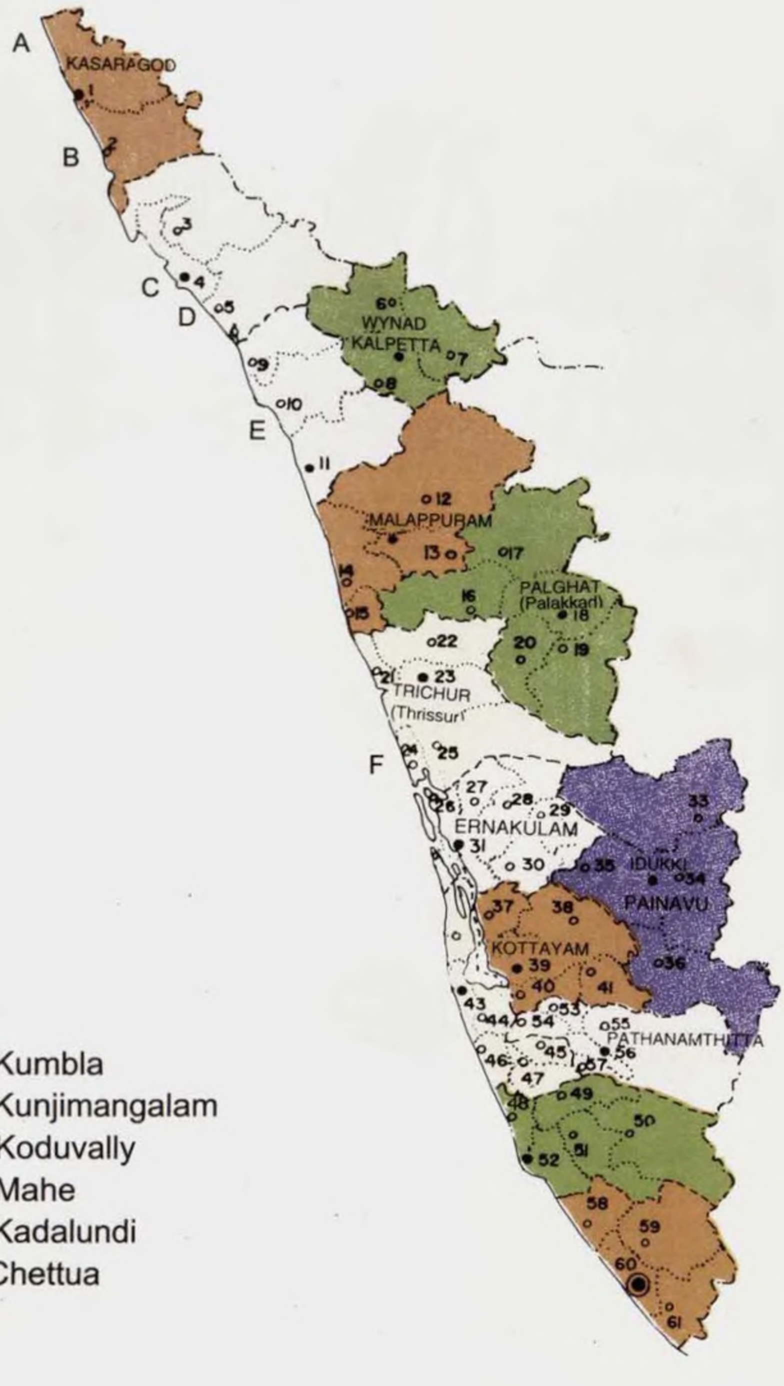
3.2. Environmental Parameters studied

Physical parameters: temperature of the particular area was noted using mercury thermometer to have an idea about the environmental conditions.

Chemical parameters: Salinity was selected as the prime supporting parameter as it has direct bearing on the estuarine condition as well as on the distribution of heavy metals and their accumulation in the biota. It was recorded using refractometer with reference to distilled water.

Dissolved oxygen was estimated by standard procedure of Strickland and Parson (1968) by using Winkler's reagent.

Biological parameter: Length of bivalves collected was noted.



A. Kumbla
B. Kunjimangalam
C. Koduvally
D. Mahe
E. Kadalundi
F. Chettua

Plate.1. Map of Kerala showing sampling stations

Plate.2. *Crassostrea madrasensis* (Indian Backwater Oyster)

Plate.3. *Saccostrea cucullata* (Indian Rock Oyster)

Plate.4. *Sunetta scripta* (Yellow clam)

3.3. Method of collection and preservation of the sample

3.3.1 Water

Water from the bar mouth area of the estuary was directly collected by immersing the clean plastic buckets. From this bucket the water was transferred to acid cleaned plastic cans of 1 litre capacity by passing through GFC filter papers of 47mm diameter.

The water samples in the plastic cans were first preserved in the ice bins and transported to laboratory and stored in deep freezer. From this, 100ml water from each site is transferred to the acid washed plastic bottles and 0.1ml of conc. HNO_3 (AR grade acid) is added to this to make the pH<2. This sample is then stored in refrigerator at approximately 4^0 C to prevent change in volume due to evaporation and stored until the time of analysis, as under these conditions samples with metal concentrations of several mg per litre are stable for up to 6months (except Hg) (APHA, 1998).

3.3.2 Sediment

Surface sediments were collected using a Van-Veen grab. Sediment samples were air dried to constant weight and ground in a ceramic grinder to pass through a sieve (of size no.85 meshes/inch). The finer sediment particles were kept air tight in polythene covers for further digestion and analysis.

3.3.3 Bivalves

Oysters (*Crassostrea madrasensis* and *Saccostrea cucullata*) and clams (*Sunetta scripta*) were manually collected from the sites, using clean knife, chisel and hammer. Oysters and clams were stored in the deep freezer after collection till the time (4-5days) of digestion.

3.4. Sample preparation and digestion

3.4.1 Tissue sample

Five to ten numbers (according to the availability) of oysters /clams of same size group were taken. Each animal was rinsed with distilled water. Soft part of the animal in its shells was rinsed with distilled water. Then the steps given below were followed:

Tissue was pooled, homogenized with a sharp scissors and mixed well in a clean watch glass.

Three grams each of the above tissue sample was taken in duplicate (Wet weight) and is dried in hot air oven at 100^0 C for 24hrs.

From this 0.25 g dry tissue was weighed and transferred to a beaker.

Ultra pure grade of HNO_3 and H_2O_2 were used for sample digestion (Martin and Flegal, 1975; Dalziel and Baker, 1983; Krishnakumar *et al.*, 1990). 10 ml of HNO_3 and H_2O_2 (digestion mixture) in1: 1 ratio was added to the sample in the beaker covered with watch glass and kept overnight. Then the beaker is placed on a hot sand bath inside a fume hood chamber at 100^0 C for 6-8hrs until the volume is reduced to 5-6ml.

Samples are removed, cooled to room temperature and transferred to a 50 ml measuring cylinder by passing through Whatman no.1 filter paper. Finally the volume is brought up to 50 ml using double distilled water. The sample is then transferred into acid cleaned plastic bottles (100 ml capacity) having labels showing center code, station code and sampling month.

3.4.2 Sediment sample

Approximately 0.5 g of air dried sediment sample is taken in glass beaker and 20 ml of digestion mixture is added. It was kept overnight and further steps are followed as in case of tissue sample. A blank solution for tissue and sediment was prepared in the same way without adding sample. Standard stock solution was prepared as per the method summerised in the working manual of AAS-Perkin Elmer model Analyst 700. Digested samples in plastic bottles are taken for heavy metal analysis in Atomic Absorption Spectrophotometer (AAS).Heavy metals (Cu, Zn, Cd and Pb) were analyzed at following wavelengths:

Cu ----324.8

Zn -----213.9

Cd ----228.8

Pb ----283.3

Mercury was analyzed in the cold vapour mercury analyzer.

3.4.3 Water sample

Fifty ml of preserved water sample was treated with 2.5ml of concentrated HNO_3 and digested at 120^0 C for 2 hours and then volume is made upto 100 ml. The digested sample was then analyzed for heavy metals Cu, Zn, Cd & Pb using 757 VA computrace (Metohm AG Herisau, Switzerland) Voltametry.

3.5. Statistical Analysis

Results were interpreted by analyzing the data statistically by Analysis of variance (ANOVA) for bivalves to study the difference in accumulation pattern between months and stations with replication, using Posthoc Least Square Difference (LSD).

Pearson correlation test was used to study the relation between the metal concentration in tissue sample and parameters like salinity, temperature, dissolved oxygen and length of the animal. The significance of the test was calculated using 'student-*t*' test with (n-2) degrees of freedom.

RESULTS

RESULTS

4. RESULTS

4.1. Station I (Kumbla)

4.1.1 Bivalves (*Saccostrea cucullata*)

The results are interpreted in Fig.1.

Copper: Average copper content of the bivalves estimated during the period of study was 152.162 ppm and the values ranged from 97.5900 ppm (Aug) to 212.4601 ppm (Nov). Statistical analysis revealed there is no significant difference in the Cu content of the bivalve tissues between months ($P > 0.05$).

Zinc: Zn accumulated in the tissues of bivalves in this station ranged from 677.1607 ppm (Dec) to 1566.693 ppm (Nov) with an average value of 889.905 ppm. Here also the values did not vary significantly between months ($P > 0.05$).

Cadmium: Accumulation of Cd in the bivalve tissues did not show any significant difference between the months ($P > 0.05$) studied. Values of Cd ranged from 8.7394 ppm (Aug) to 37.9383 ppm (Nov) with an average value of 23.042 ppm. Mercury: In the case of mercury accumulation in the bivalve tissues, significant difference was observed between the months ($P < 0.05$). Mercury content in this station ranged from 0.2985 µg/g (Dec) to 2.2253 ppm (Sept). Difference was more between November and December when compared to other months, with less value.

Lead concentrations in the tissues were found below the detectable levels throughout the study period.

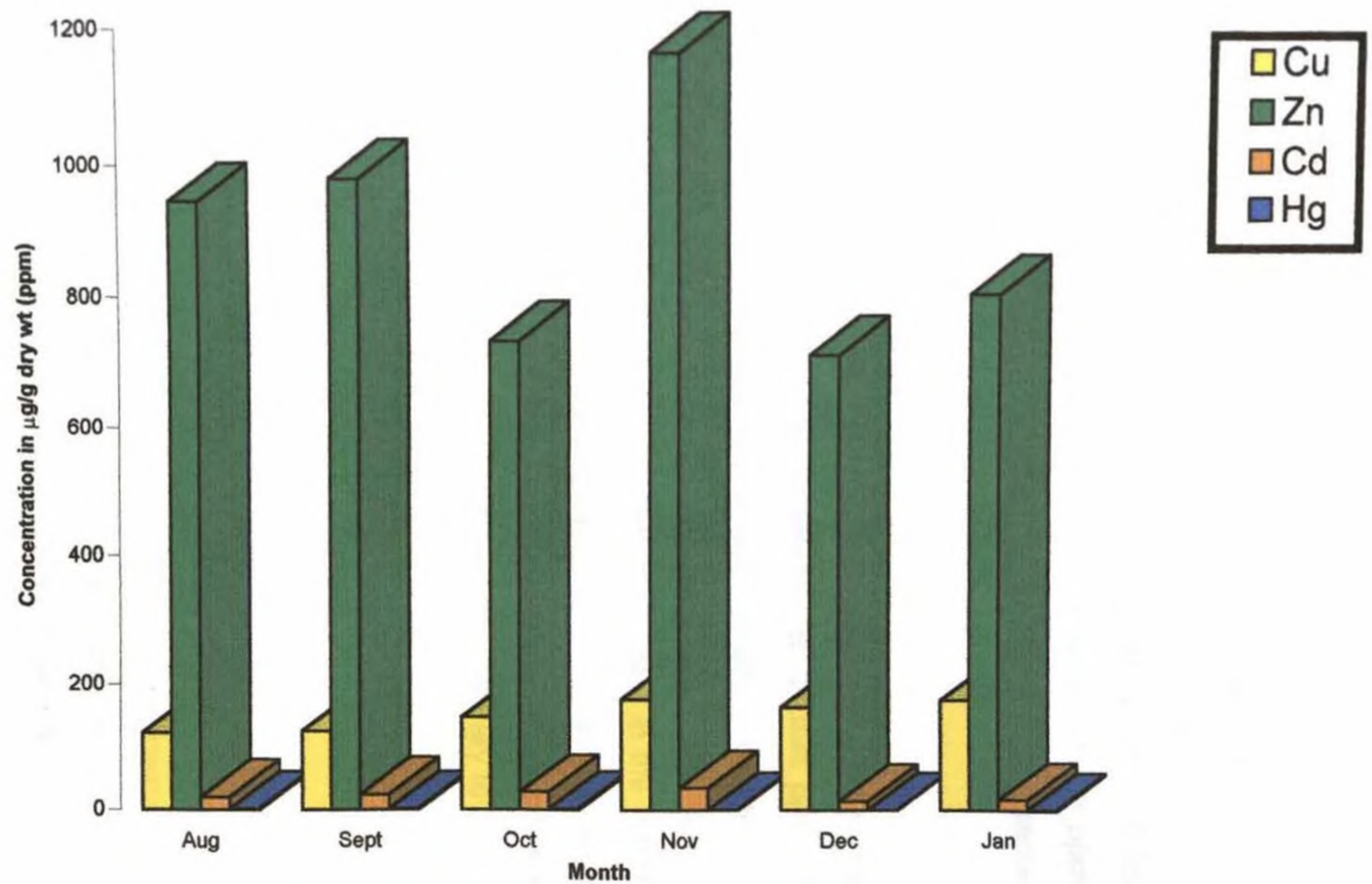


Fig.1 Heavy Metal Content in Bivalves of Station I

4.1.2 Sediments

Copper content in the sediment ranged from 1.288 ppm (Sept) to 8.0693 ppm (Nov) with an average concentration of 3.1997 ppm. Where as Zinc content ranged from 4.996 ppm (Jan) to 362.676 ppm (Dec) with an average of 69.1043 ppm. Average cadmium concentration was found 6.887 ppm with the values ranging from 0.6969 ppm (Aug) to 19.625 ppm (Nov). Concentration of mercury ranged from 0.0507 ppm (Nov) to 0.353 ppm (Oct) with an average value of 0.1992 ppm. Lead content in the sediment sample of this station was below detectable limit (Fig.2).

4.1.3 Water

Concentration of copper in the water from the same station was found ranging from 0.060 ppm (January) to 21.836 ppm (October) with an average value of 10.451 ppm. Average zinc concentration in the water was 0.712 ppm. And the concentration ranged from 0.457 ppm in January to 0.885 ppm in October. Cadmium content in the water ranged from 0.002 ppm in January to 0.005 ppm in November with an average concentration of 0.003 ppm. Average concentration of lead was found 0.205 ppm with values ranging from 0.165 ppm in January to 0.256 ppm in November (Fig.3).

4.1.4 Other Parameters

Apart from the heavy metal contents studied from the bivalve tissues; parameters like Salinity, Temperature, and Dissolved oxygen of the water from different stations were studied. Correlations between accumulations of heavy metals in bivalves with the water salinity, temperature were also studied. Salinity ranged from 1.67 ppt in August to 29.6 ppt in January with an average value of 14.798 ppt. Average value of temperature noted 30.5^0 C with a range from 28.2^0 C (Sept) to 32.5^0 C (Oct) respectively. Average dissolved oxygen content was 4.139 ml/l and values ranged from 3.058 ml/l (Sept) to 4.96 ml/l (Aug). Length of the animal (*Saccostrea cucullata*) used for the pollution studies from this station were with an average length 4.4 cm. (Fig.4).

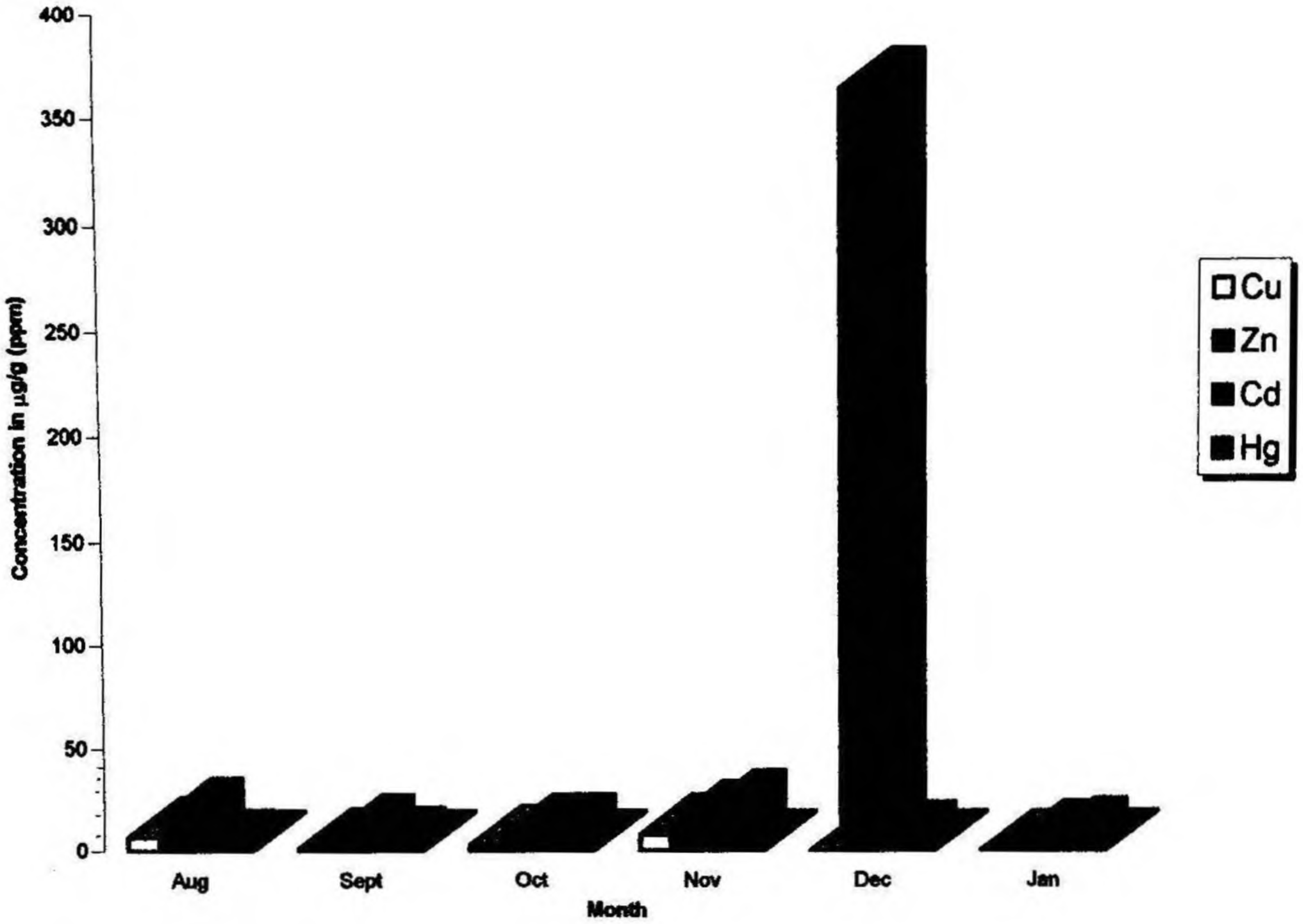


Fig.2 Heavy Metal Content in Sediment of Station I

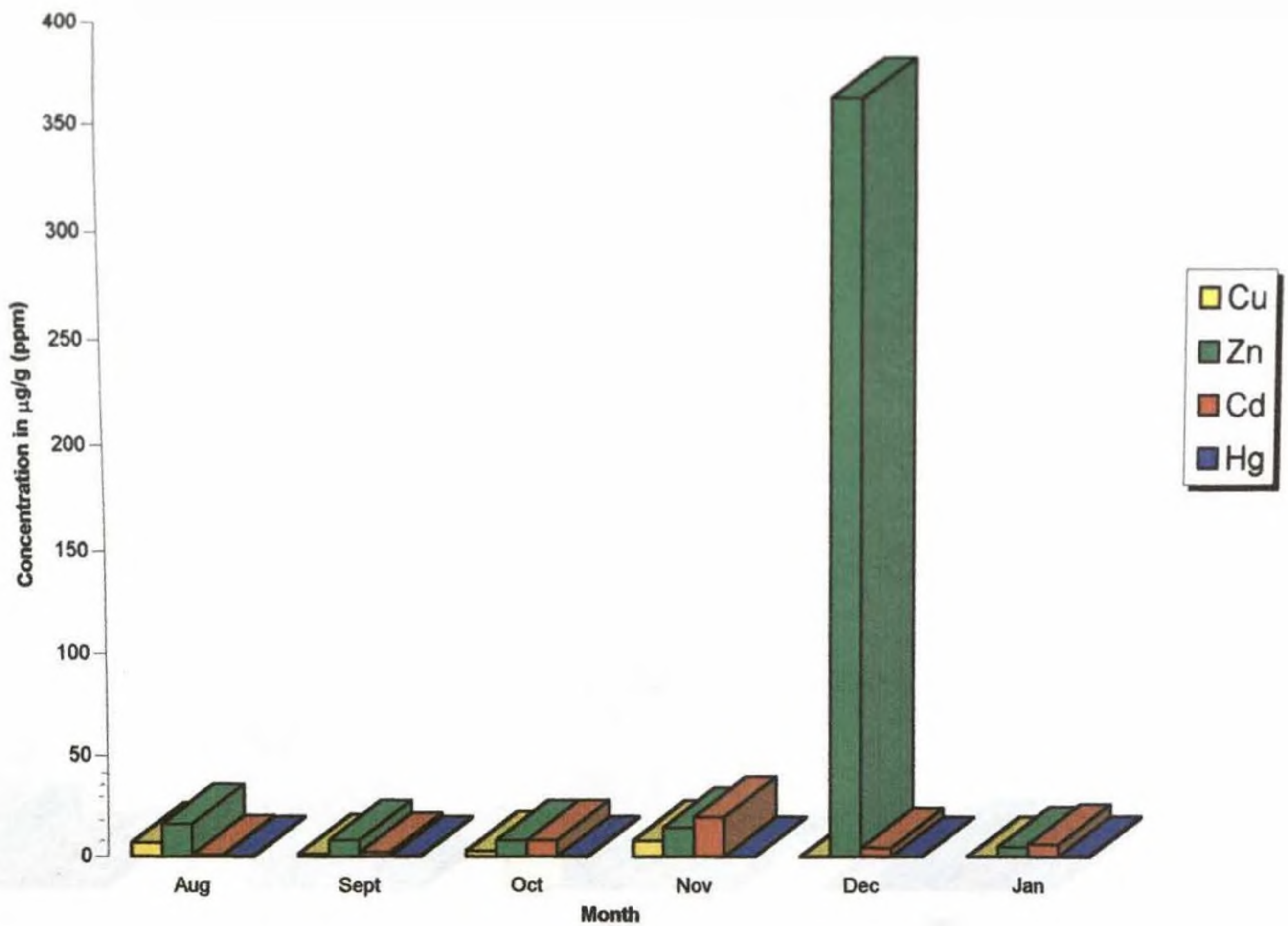


Fig.2 Heavy Metal Content in Sediment of Station I

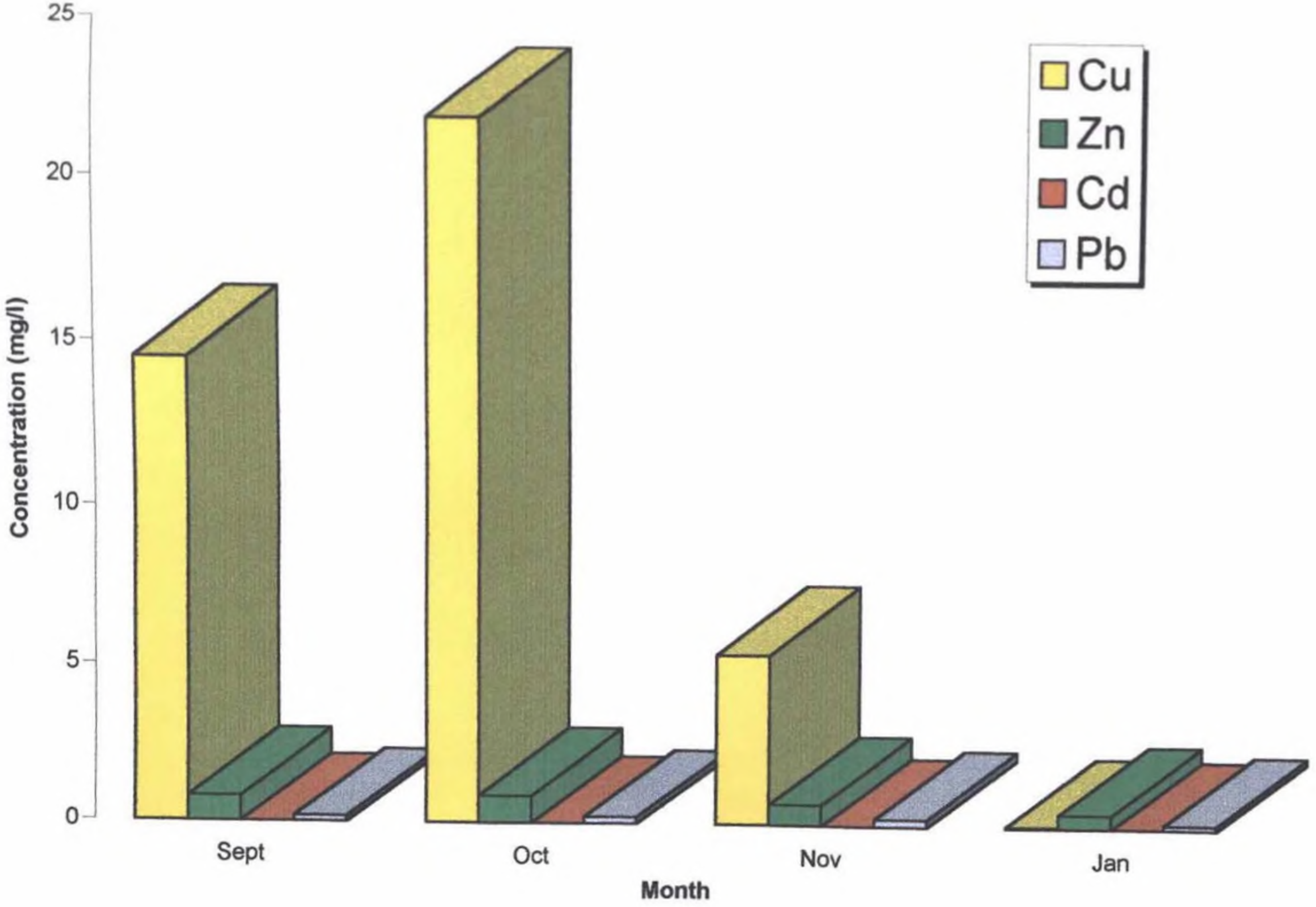


Fig.3 Heavy Metal Content in Water of Station I

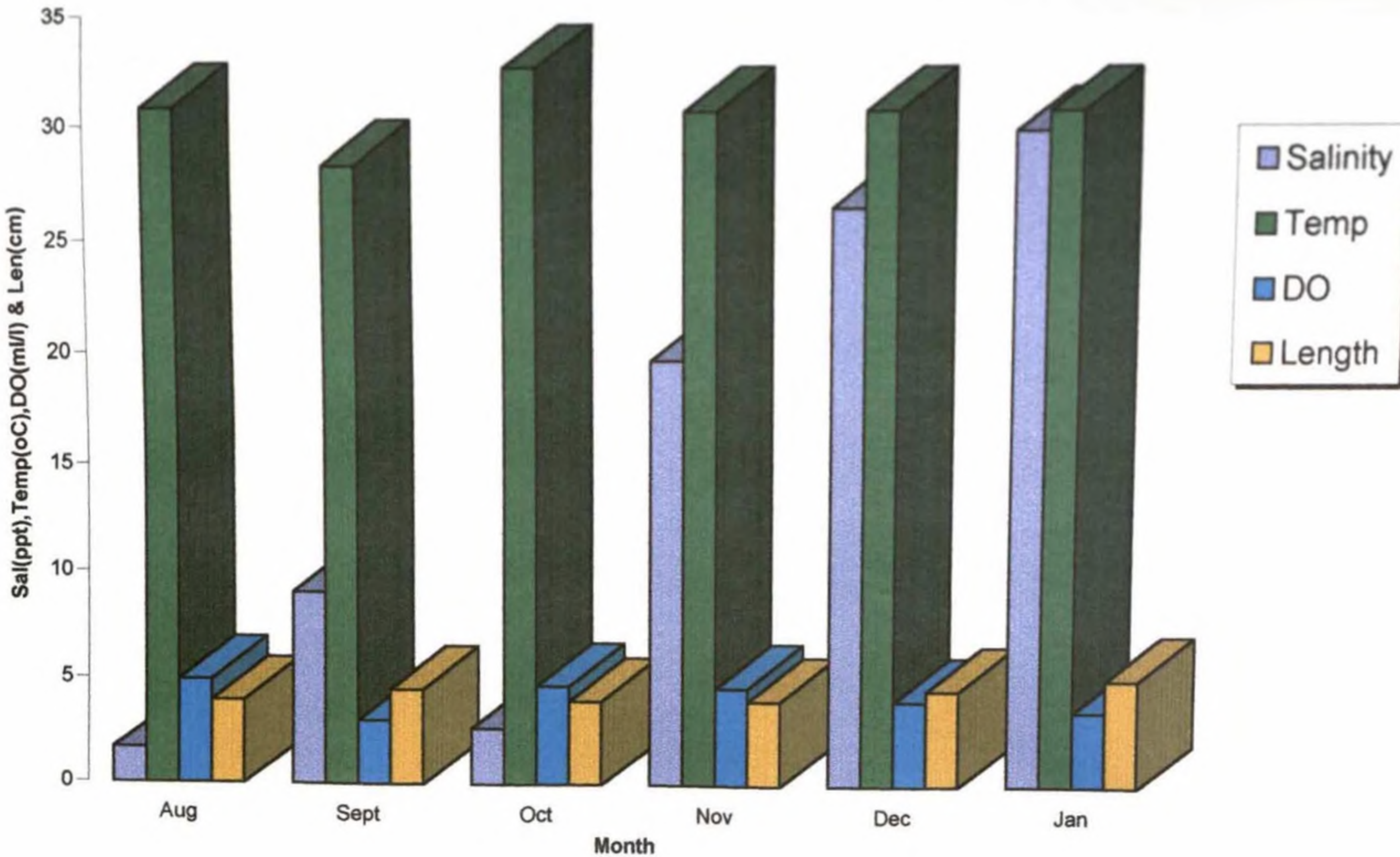


Fig.4 Parameters of Station I

4.2. Station II (Kunjimangalam)

4.2.1 Bivalves (*Saccostrea cucullata*)

The results are interpreted in Fig.5.

Copper: Cu in the bivalve tissues ranged from 19.5 ppm (Aug) to 217.5021 ppm (Nov) with an average value of 124.152 ppm. The values showed significant difference between the months ($P<0.05$). Difference was mainly noticed in November and December with a higher concentration than in other months.

Zinc: Zn content accumulated in the tissues ranged from 351.1 ppm (Aug) to 1808.195 ppm (Dec) with an average concentration 1111.206 ppm. There was no significant difference in the Zn content values between months during the study period ($P>0.05$).

Cadmium: Cd concentration in the tissues showed significant difference between months ($P<0.05$). Difference was seen among all months except between September and October where the accumulated content is almost same. The values of Cd ranged from 3.5(Aug) to 42.2684 (Nov) with an average content of 20.5950 ppm.

Mercury: Mercury content in the tissues also showed significant difference. Difference was mainly seen in September, November, December and January with lower content compared to August and October. Mercury content ranged from 0.0999 ppm (Jan) to 1.5532 ppm (Oct) with average content of 0.786 ppm. Lead content in the tissues were analyzed and found that it was much below the detectable level.

4.2.2 Sediments

Copper: Cu content ranged from 0.198 ppm (Oct) to 4.7737 ppm (Aug) with an average value of 1.5912. Average Zn content was found 30.021 ppm with the values ranging from 4.270 ppm (Oct) to 127.035 ppm (Dec). Cadmium content ranged from 0.248 ppm (Aug) to 9.353 ppm (Nov) with an average value of 4.651 ppm. Mercury average Hg content was found to be 0.2159 ppm with the values ranging from 0.0302 ppm (Dec) to 0.5038 ppm (Sept). Lead content in the sediments during the study period was found much below the detectable level (Fig.6).

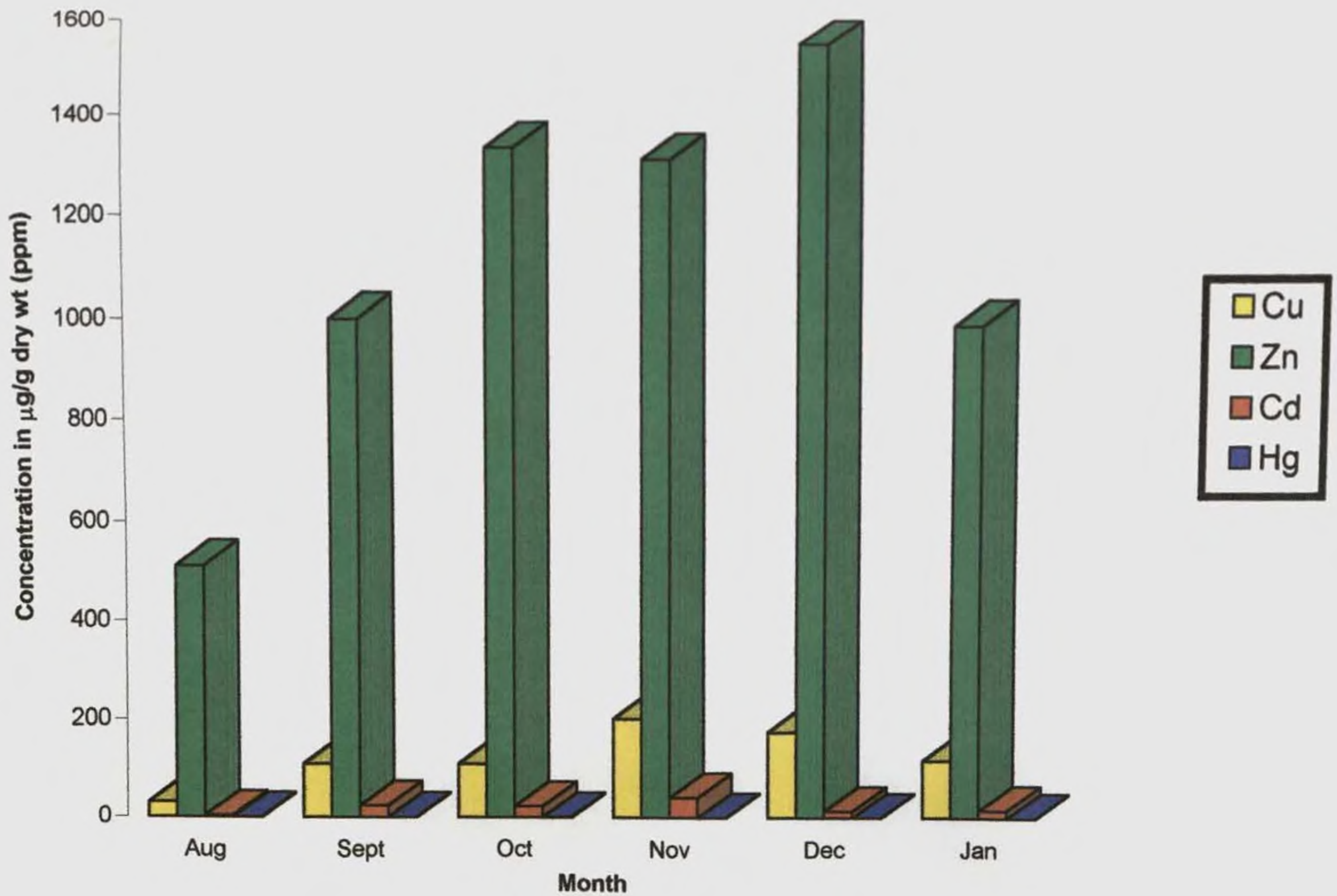


Fig.5 Heavy Metal Content in Bivalves of Station II

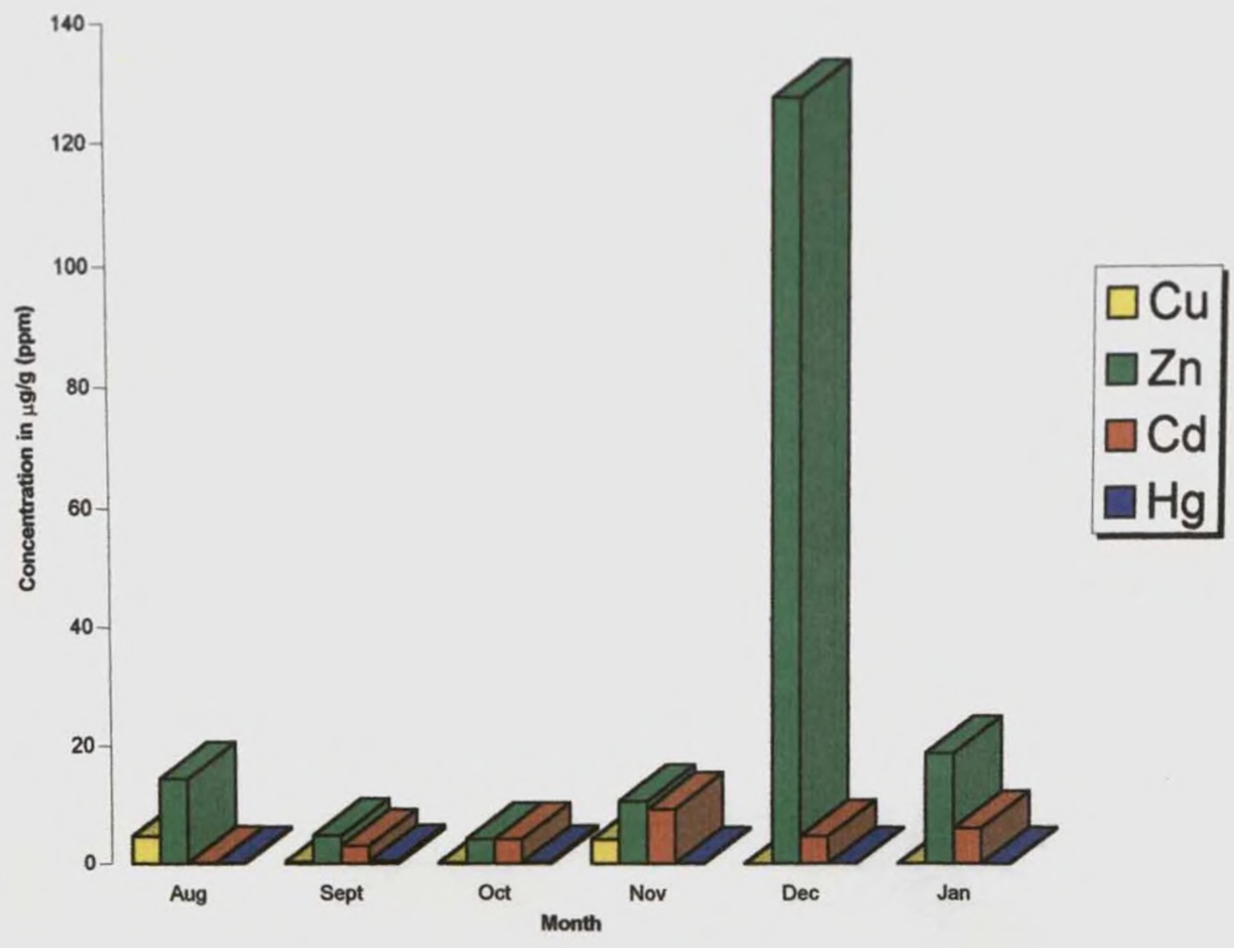


Fig.6 Heavy Metal Content in Sediment of Station II

4.2.3 Water

Average content of copper in water was 4.836 ppm. Highest copper content observed was 12.392 ppm (October) and the lowest value obtained was 0.104 ppm (January). Range of Zinc content was between 0.382 ppm (January) and 1.302 ppm (October) with an average value of 0.720 ppm. Cadmium content in the water was found ranged from 0.002 ppm (September) to 0.032 ppm (October). Average concentration found during the study period was 0.010 ppm. Lead content ranged from 0.092 ppm in September to 0.442 ppm in October with an average value of 0.220 ppm (Fig.7).

4.2.4 Other Parameters

Salinity ranged from 0.5 ppt in August to 27.37 ppt in December. Average value was found to be 13.933 ppt. Temperature ranged from 28^0 C in August to 32.8^0 C in September. Dissolved oxygen from 2.356 ml/l (Oct) to 4.914 ml/l (Sept) with an average value of 4.0911 ml/l. Length of the animals (*Saccostrea cucullata*) used for the bioaccumulation study in this station ranged from 4.5 cm and 7 cm with an average length of 5.25 cm (Fig.8).

4.3. Station III (Mahe)

4.3.1 Bivalves (*Crassostrea madrasensis*)

The results are interpreted in Fig.9.

Copper: Copper content in the bivalve tissues during the study period in this station ranged from 16.487 ppm (Jan) to 172.967 ppm (Sept) with an average value of 81.291 ppm. It showed significant difference between months ($P<0.05$). Significant difference was seen in December and January months with lower values compared to other months.

Zinc: Average Zn content was 1172.264 ppm with the values ranging from 434.843 ppm (Feb) to 1951.632 ppm (Oct). The values showed significant difference between months ($P<0.05$). Significant difference was noticed in December and January with lower values.

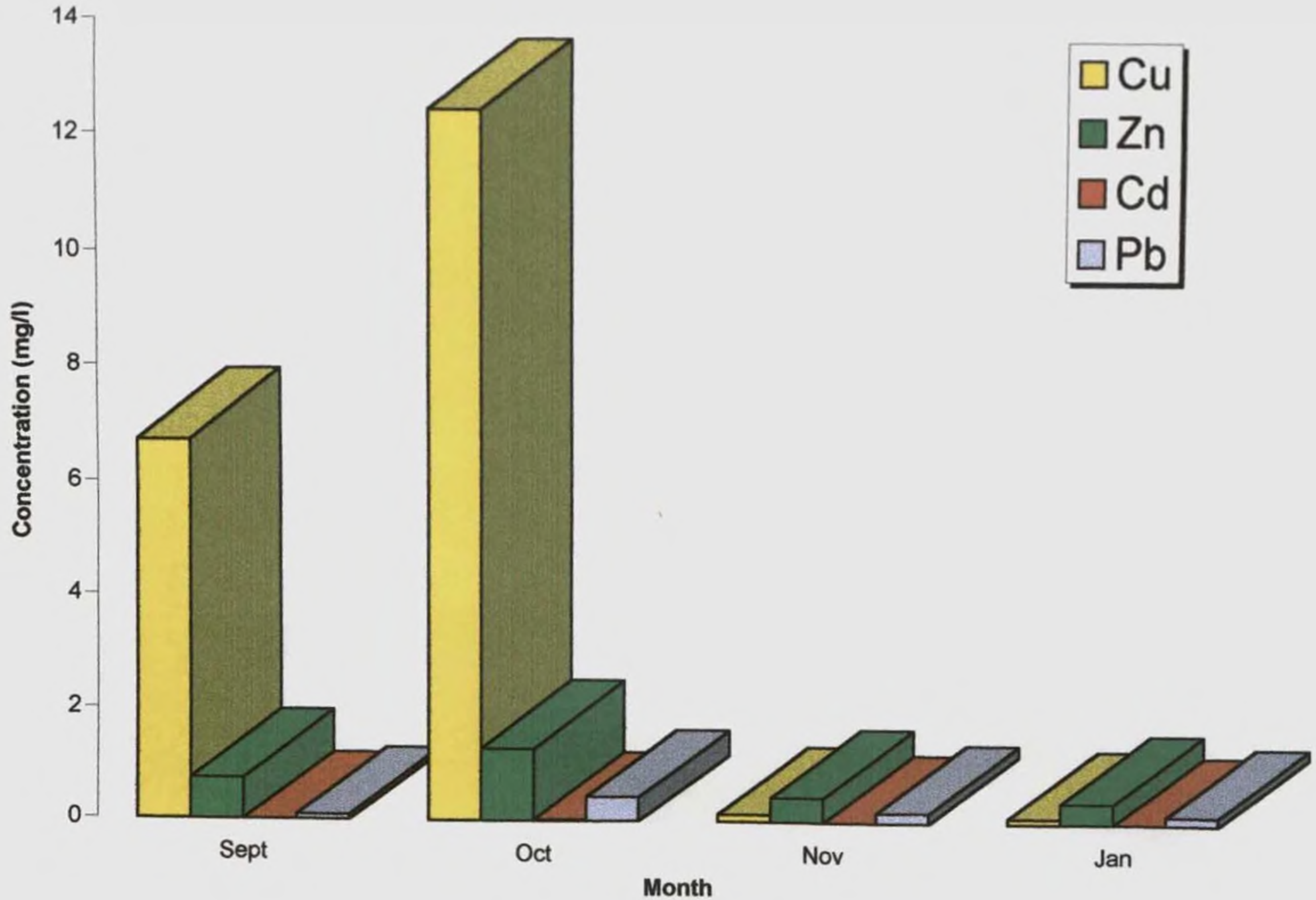


Fig.7 Heavy Metal Content in Water of Station II

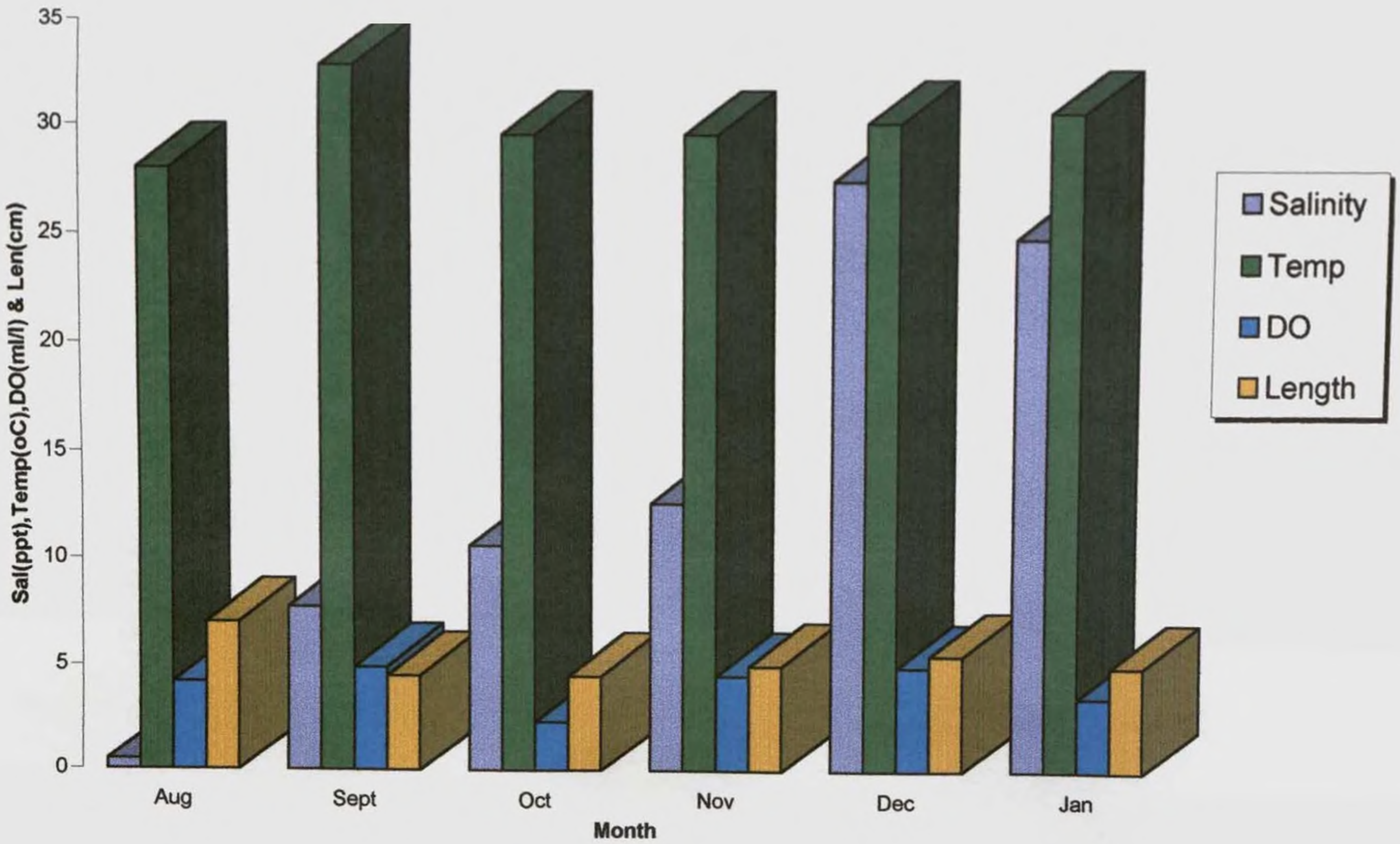


Fig.8 Parmeters of Station II

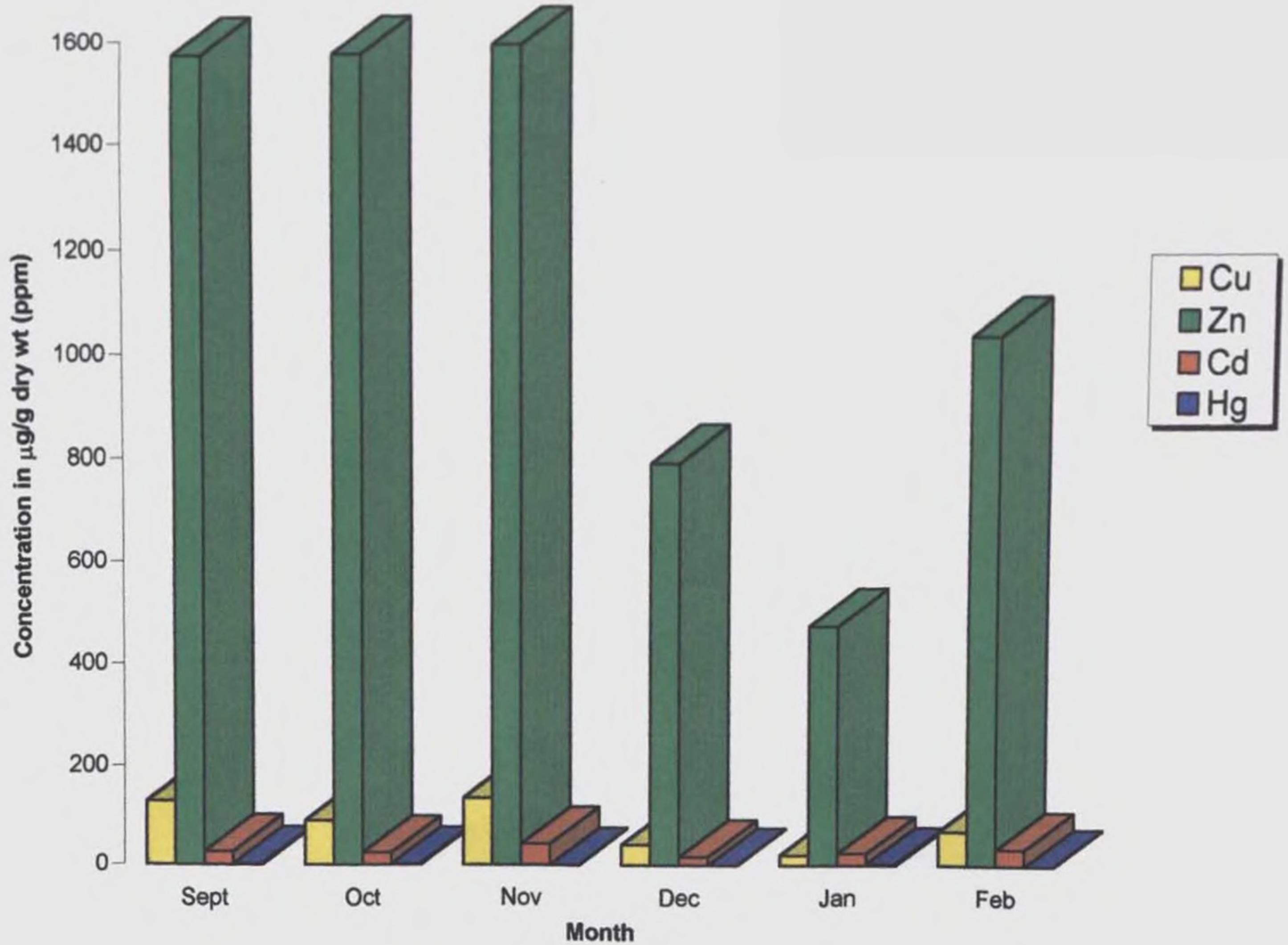


Fig.9 Heavy Metal Content in Bivalves of Station III

Cadmium: Cd content ranged from 14.040 ppm (Dec) to 43.841 ppm (Nov) with an average value being 28.281 ppm. Significant difference was found in the Cd contents between months ($P < 0.05$). Significant difference was noticed in November, December and February months with higher values.

Mercury: Average mercury content was 1.121 ppm with the values ranging from below detectable level (Feb) to 4.6217 ppm (Jan). There was no significant difference in the values between the months.

Lead content in the tissues studied was found much below the detectable level.

4.3.2 Water

Average copper content in the water from the station III was 10.781 ppm. Cu content during the study period ranged from 0.048 ppm to 42.518 ppm in November and September months respectively. The average zinc content of the water was found to be 0.590 ppm with the values ranging from 0.388 ppm (Nov) to 0.856 ppm (Oct). Cadmium content in this water ranged from 0.001 ppm to 0.006 with an average of 0.0027 ppm (Fig.10).

4.3.3 Other Parameters

Values of salinity ranged from 24 ppt (Sept) to 30.5 ppt (Oct) with average value of 27.08 ppt. Temperature ranged from 27.4^0 C (Sept) to 29.6^0 C (Nov) with an average of 28.31^0 C. Length of animals (*Crassostrea madrasensis*) used for the bioaccumulation studies ranged from 8 cm to 11.5 cm with an average of 9.0 cm (Fig.11).

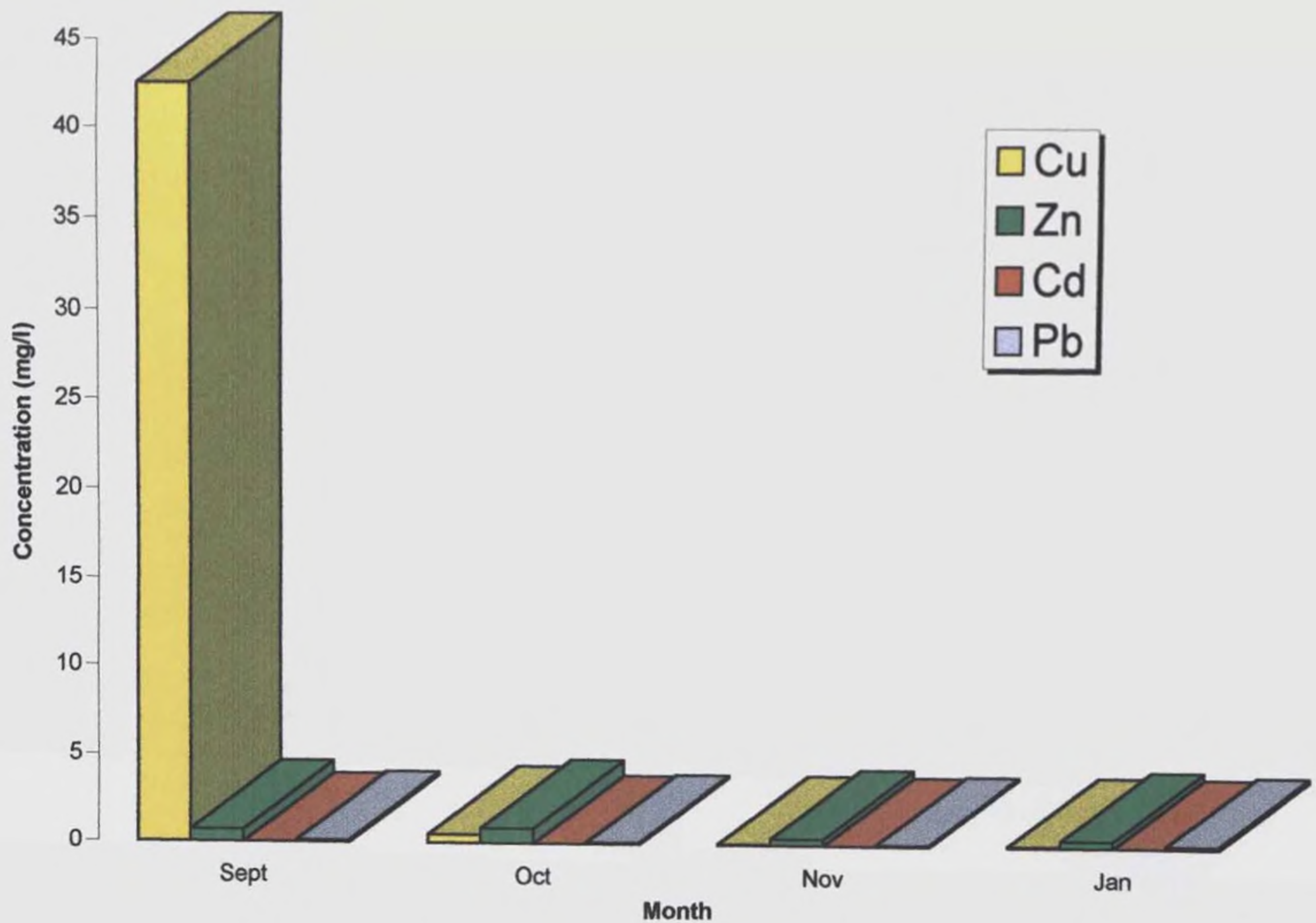


Fig.10 Heavy Metal Content in Water of Station III

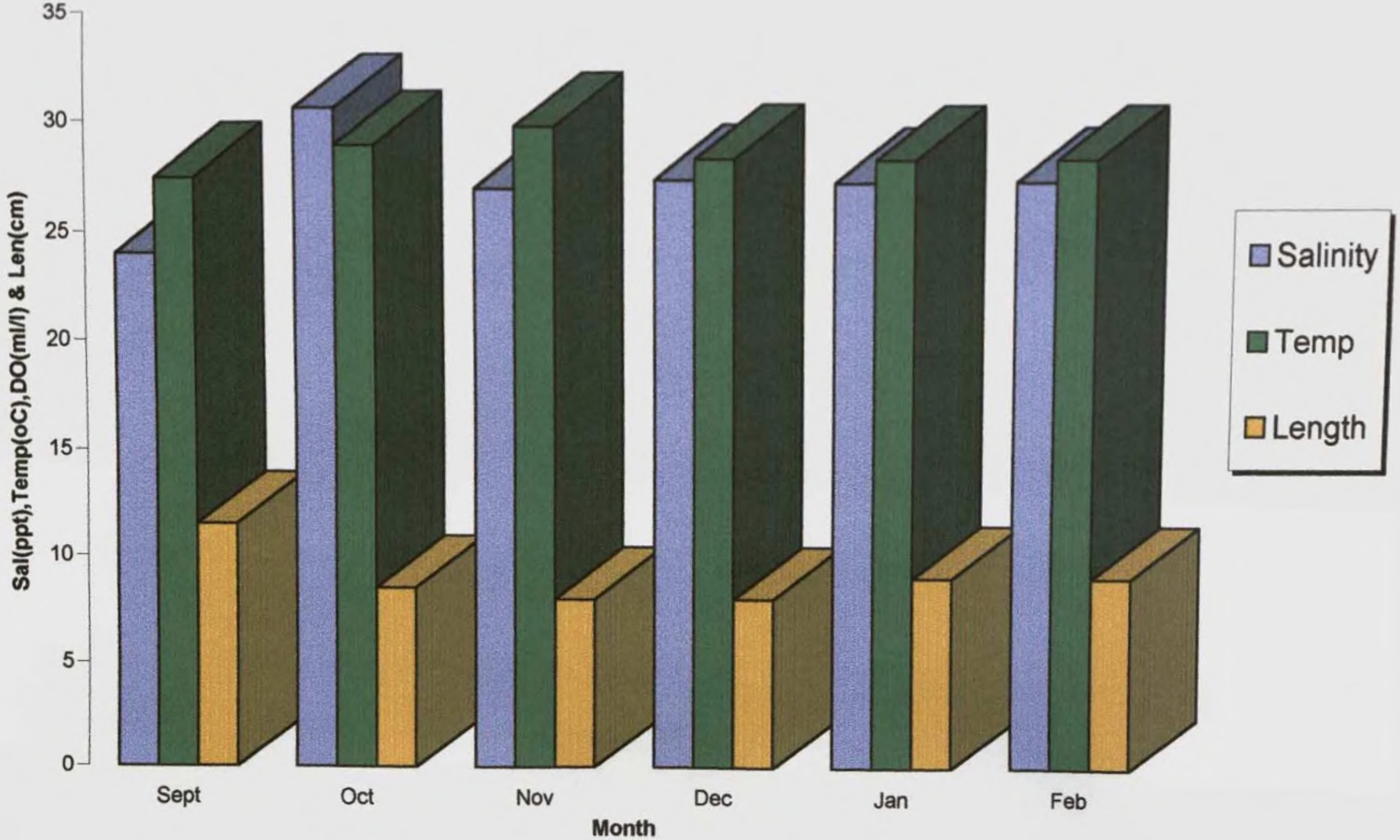


Fig.11 Parameters of Station III

4.4. Station IV (Kadalundi)

4.4.1 Bivalves (*Crassostrea madrasensis*)

The results are interpreted in Fig.12.

Copper: Concentration ranged from 6.67786 ppm (Aug) to 31.38318 (Nov) ppm with an average value of 18.88 ppm. It showed significant difference between months ($P<0.05$). The highest value noticed in November compared to other months.

Zinc: Average value of zinc was 644.8098 ppm with values ranging from 249 ppm (Sept) to 1566.447 ppm (Nov). Zn showed significant difference between months. Difference was not seen in August and September, which showed lower values than other months.

Cadmium: Concentration of Cd ranged from 8.7175 ppm (Nov) to 28.899 ppm (Oct) with an average value of 20.6537 ppm. It showed significant difference between months. Difference was not noticed in August and September with lower values.

Mercury: Average mercury concentration was found to be 1.146095 ppm with the values ranging from 0 ppm (Jan) to 2.8995 ppm (Aug). It showed significant difference between months ($P<0.05$). Differences in the values were noticed in all months except August and November, which showed higher values.

Lead content in the tissues studied was found much below the detectable level.

4.4.2 Sediment

Concentration of copper ranged from 2.595 ppm (Dec) to 23.083 ppm (Nov) with an average value of 13.145 ppm. Concentration of zinc ranged from 0.057 ppm (Oct) to 105.675 ppm (Dec) with an average value of 58.778 ppm. Average concentration of cadmium was found to be 5.676 ppm with the values ranging from 0.039 ppm (Oct) to 12.7798 ppm (Nov). Mercury content ranged from 0.050 ppm (Nov) to 0.5076 ppm (Jan) with an average content of 0.210 ppm. Lead was found much below detectable level in the sediments of station IV (Fig.13).

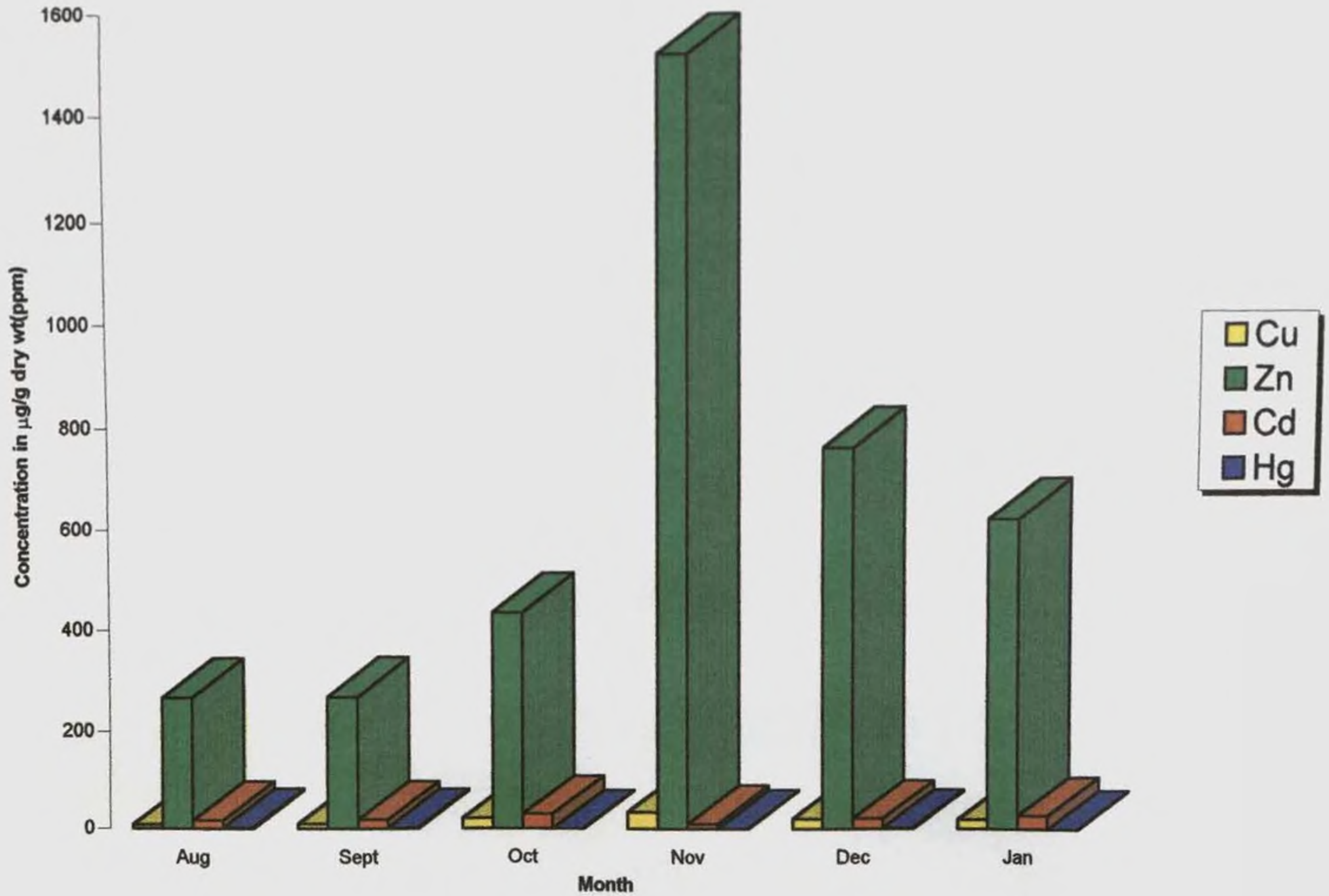


Fig.12 Heavy Metal Content in Bivalves of Station IV

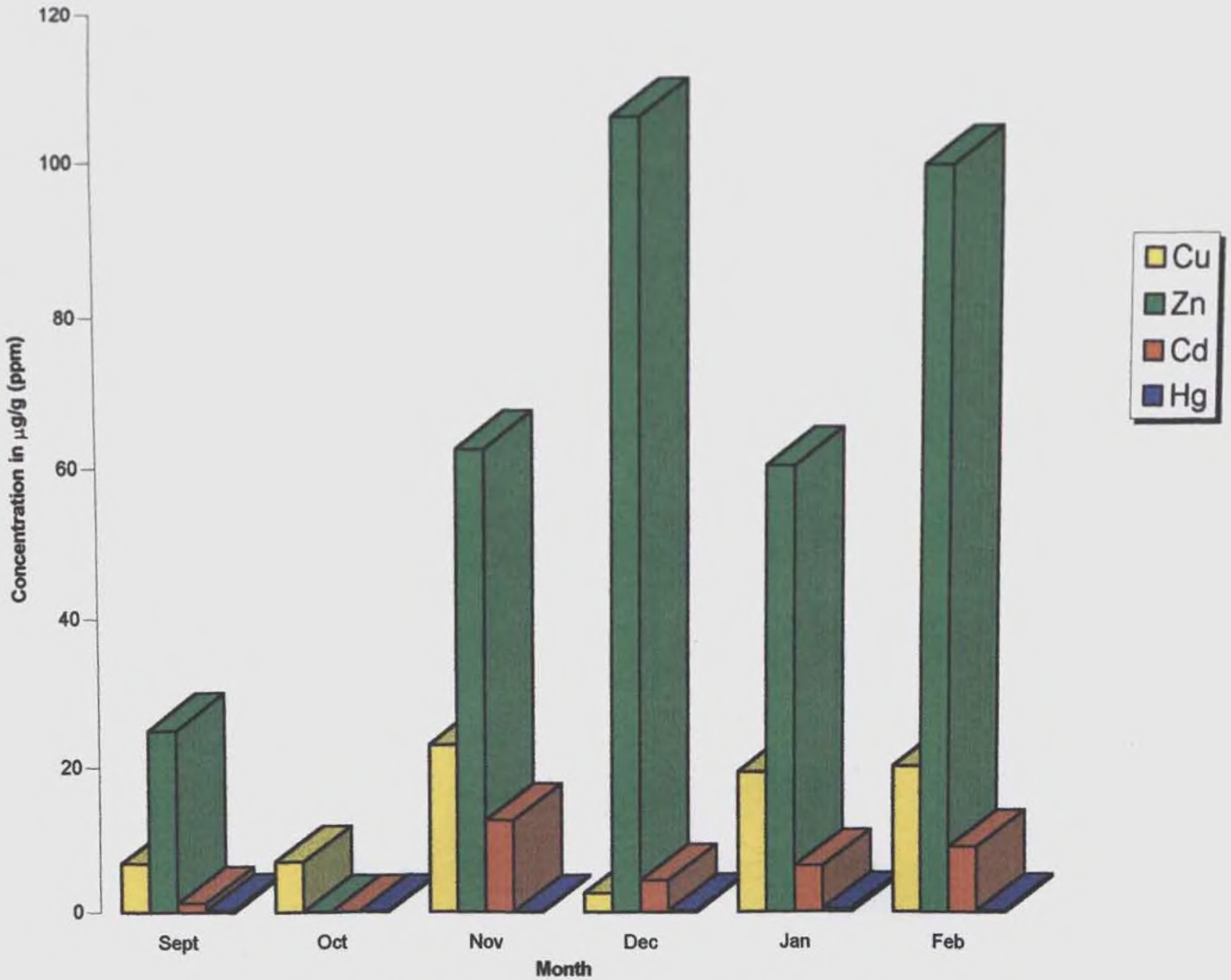


Fig.13 Heavy Metal Content in Sediment of Station IV

4.4.3 Water

Concentration of copper ranged from 0.279 ppm (Jan) to 14.872 ppm (Oct) with an average value of 6.36 ppm. Average concentration of zinc was found to be 1.144 ppm with the values ranging from 0.378 ppm (Jan) to 1.719 ppm (Nov). Concentration of cadmium ranged from 0.005 ppm (Jan) to 0.010 ppm (Nov) with an average value of 0.0075 ppm. Values of lead ranged from 0.101 ppm (Sept) to 0.333 ppm (Nov) with an average of 0.193 ppm (Fig.14).

4.4.4 Other Parameters

The salinity of the water from this varied from 1.18 ppt (Oct) to 30.72 ppt (Jan) during the study period. The average salinity was found 15.806 ppt. Temperature of the water ranged from 29^0 C to 30.5^0 C with an average value of 29.46^0 C. Dissolved oxygen contents of the water in the station was found ranged from 4.116 ml/l (Nov) to 6.279 ml/l (Sept) with an average value of 5.08 ml/l. Length of the animal(*Crassostrea madrasensis*) used for the pollution studies ranged from 3.5 cm (Aug) to 9.5 cm (Dec) with an average value of 7.66 cm (Fig.15).

4.5.Station V (Koduvally)

4.5.1 Bivalves (*Crassostrea madrasensis*)

The results are interpreted in Fig.16.

Copper: Cu content ranged from 23.181 ppm (Oct) to 107.001 ppm (Dec), with an average concentration of 47.315 ppm. It showed significant difference between months ($P<0.05$). Difference was mainly seen in December with higher value.

Zinc: Values of Zn ranged from 372.850 ppm (Sept) to 1272.152 ppm (Nov) with an average value of 777.957 ppm. It showed significant difference and this was mainly seen in November with higher value.

Cadmium: Average content of Cd was found to be 21.059 ppm with the values ranging from 7.796 ppm (Sept) to 31.126 ppm (Nov) respectively. It did not show significant difference between months.

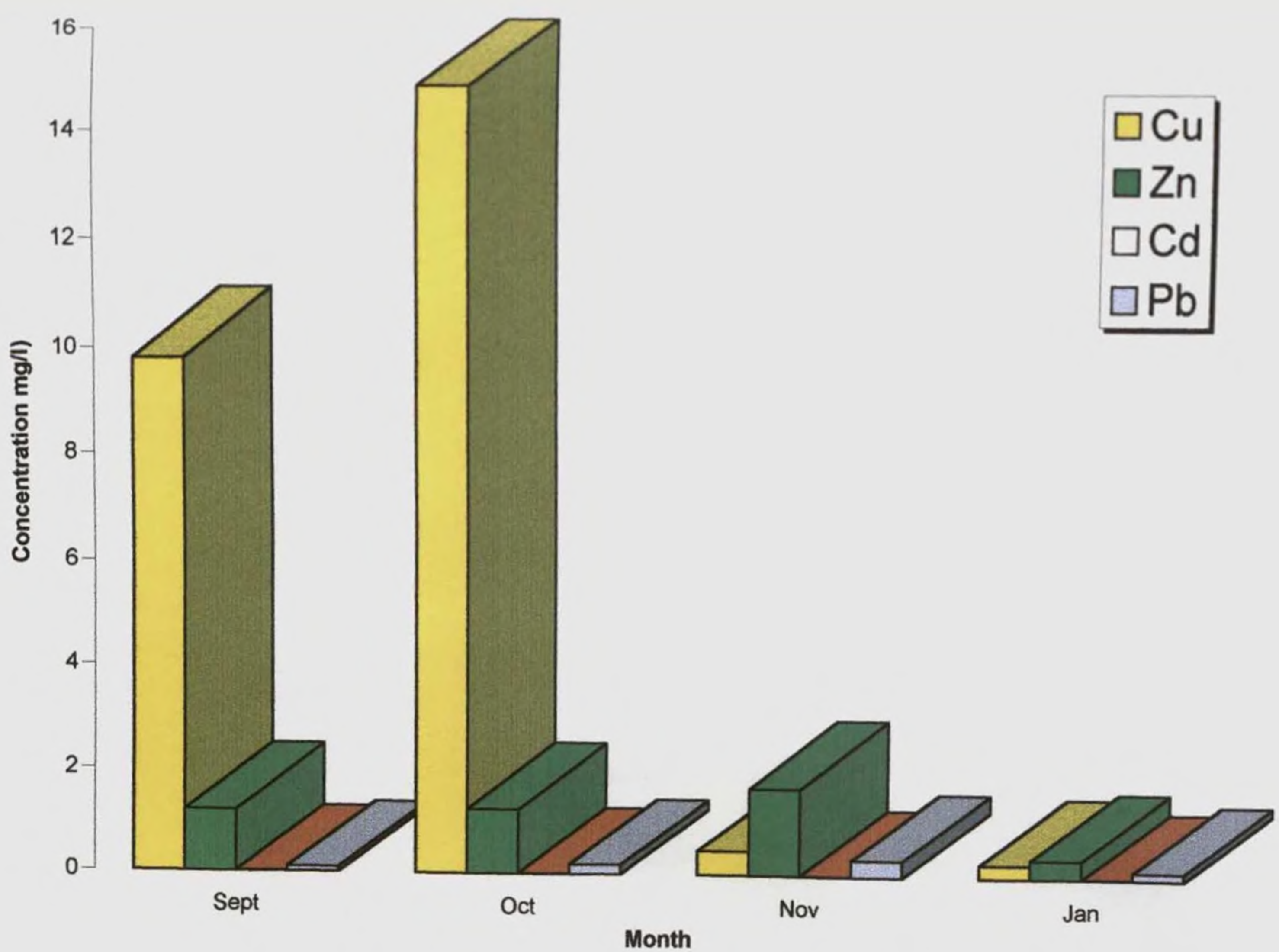


Fig.14 Heavy Metal Content in Water of Station IV

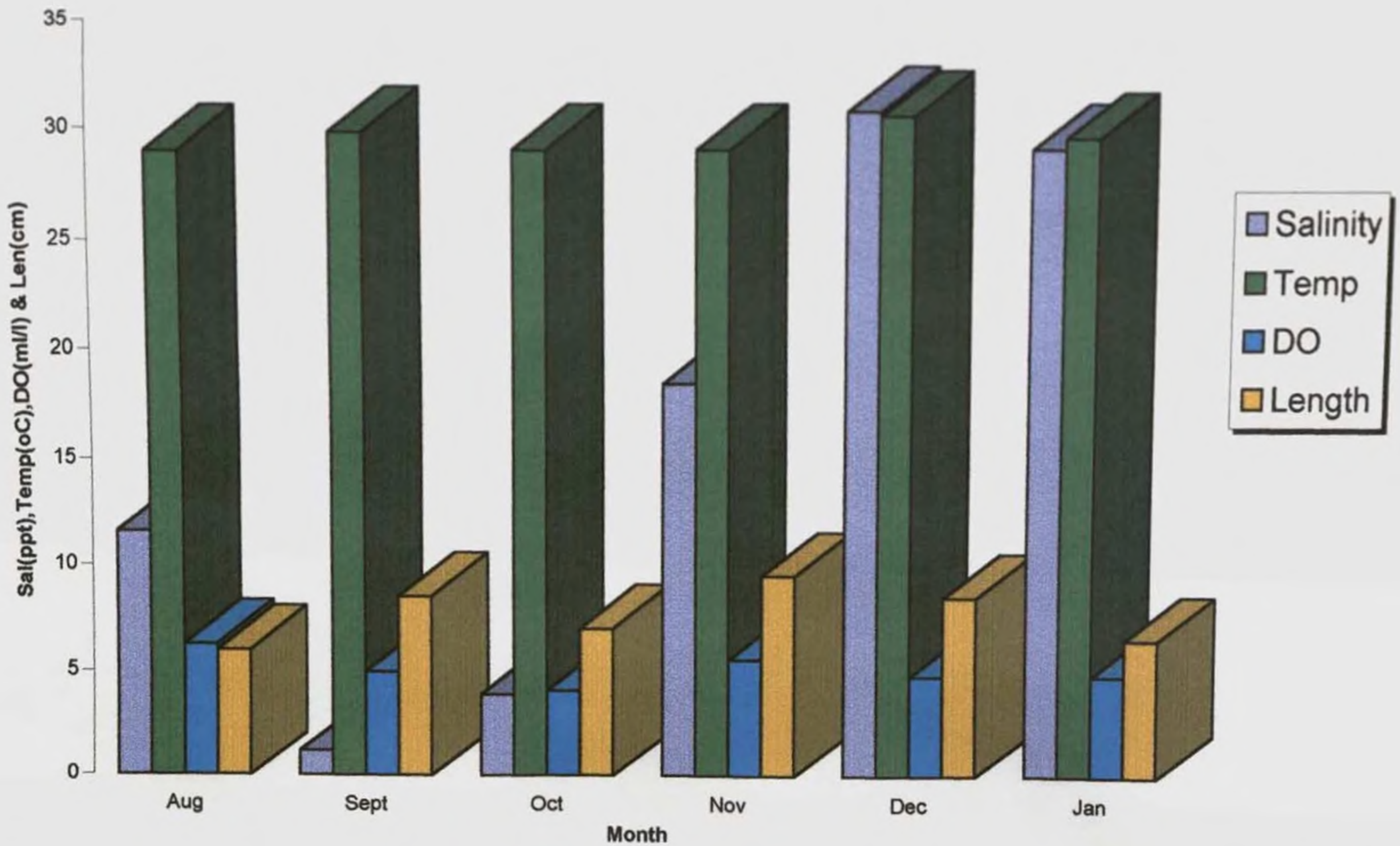


Fig.15 Parameters of Station IV

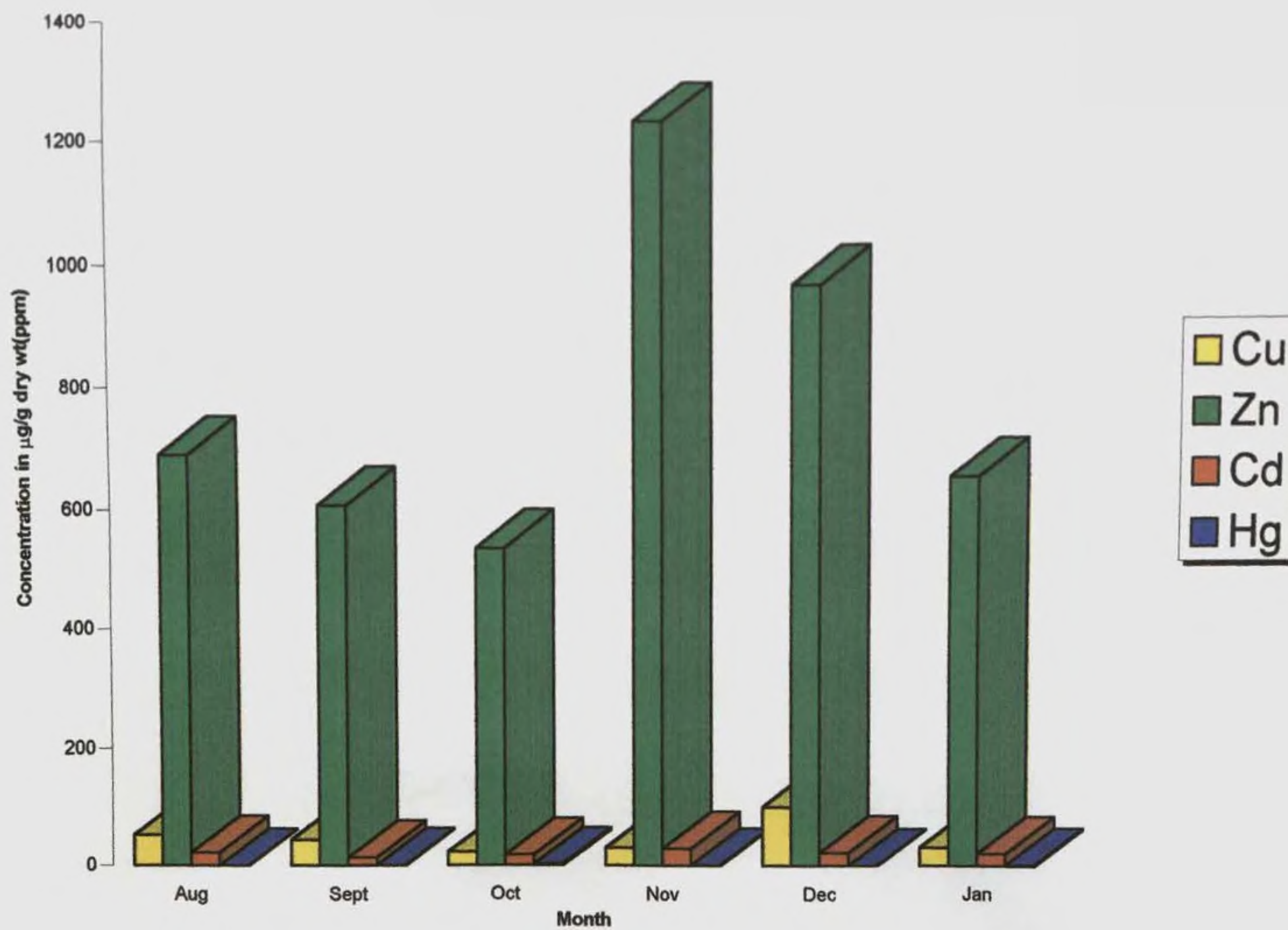


Fig.16 Heavy Metal Content in Bivalves of Station V

Mercury Values of Hg ranged from 0.10 ppm (Dec) to 2.136 ppm (Oct) with an average of 0.837 ppm. It showed significant difference between months ($P < 0.05$). Difference was mainly noticed due to higher values in October and January.

4.5.2 Sediments

Copper content ranged from 0.995 ppm (Oct) to 27.253 ppm (Nov) with an average value of 10.955 ppm. Zinc content ranged from 5.075 ppm (Oct) to 213.235 ppm (Dec) with an average value of 66.204 ppm. Cadmium content ranged from 0.049 ppm (Aug) to 8.953 ppm (Nov) with an average value of 4.43 ppm. Mercury content ranged from 0.100 ppm (Nov) to 8.4132 ppm (Aug) with an average content of 3.0913 ppm (Fig.17).

4.5.3 Water

Copper content ranged from 0.152 ppm (Nov) to 10.798 ppm (Oct) with an average of 4.345 ppm. Zinc content ranged from 0.580 ppm (Jan) to 3.282 ppm with an average value of 1.380 ppm. Cadmium content ranged from 0.002 ppm to 0.023 ppm with an average value of 0.0097 ppm. Lead content ranged from 0.110 ppm (Sept) to 1.027 ppm (Nov) and with an average value of 0.335 ppm (Fig.18).

4.5.4 Other Parameters

Average salinity noticed was 18.841 ppt with the values ranging from 14.2 ppt (Aug) to 28.86 ppt (Jan) respectively. Temperature ranged from 23.8^{0} C (Sept) to 30.5^{0} C (Jan) with an average of 28.1^{0} C. Average Dissolved oxygen obtained was 3.931 ml/l with the values ranging between 1.802 ml/l (Sept) and 4.954 ml/l (Dec). Length of the animals (*Crassostrea madrasensis*) used for the bioaccumulation studies varied between 7 cm (Aug) and 11 cm (Oct, Nov) with an average length of 9.3 cm (Fig.19).

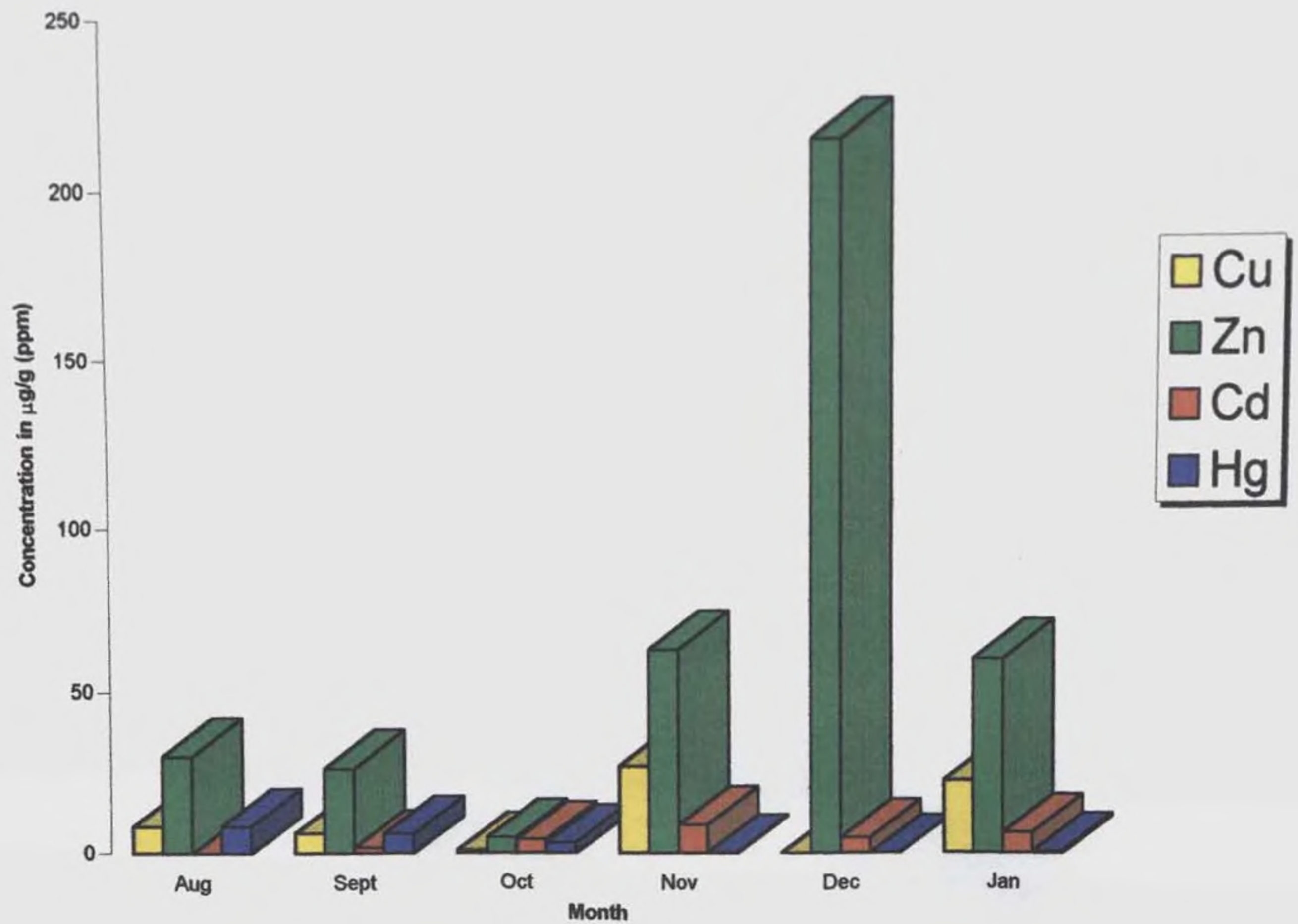


Fig.17 Heavy Metal Content in Sediment of Station V

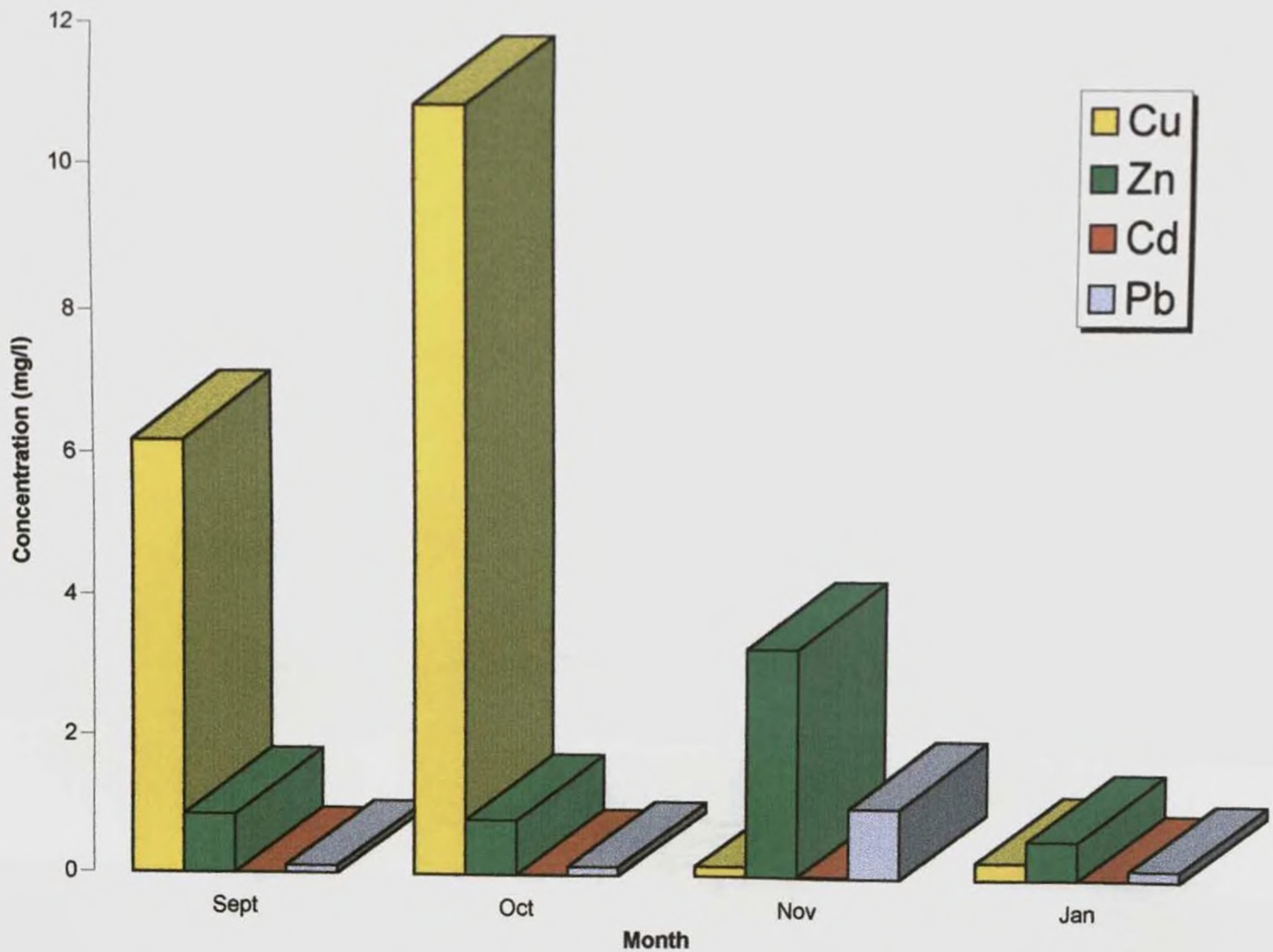


Fig.18 Heavy Metal Content in Water of Station V

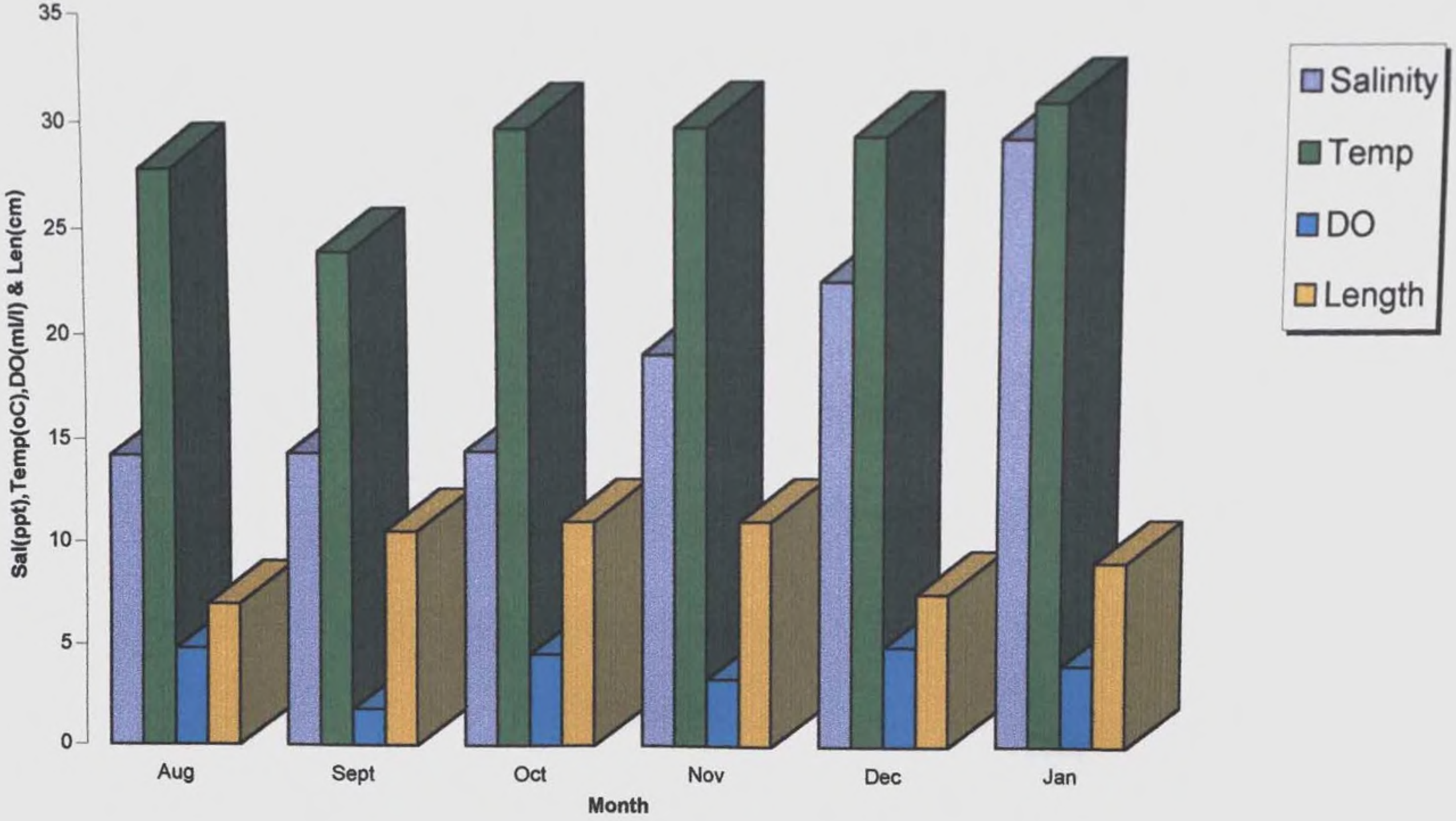


Fig.19 Parameters of Station V

4.6. Station VI (Chettua)

4.6.1 Bivalves (*Sunetta scripta*)

The results are interpreted in Fig.20.

Copper: Copper content in the tissues of clams studied ranged from 2.99 ppm to 16.003 ppm with an average content of 6.956 ppm. It showed significant difference between months ($P<0.05$) and difference was seen in January and February with the concentration below the detectable level.

Zinc: Zinc content ranged from 40.6746 ppm to 122.106 ppm with an average content of 74.3028 ppm. It showed significant difference between months ($P<0.05$) with lower content in December.

Cadmium: Average content of Cd was found to be 20.5496 ppm in the tissues with the values ranging from 8.889 ppm to 31.723 ppm. Cd also showed significant difference between months ($P<0.05$), with lower concentrations in January and February.

Mercury: Hg content ranged from 0.8044 ppm to 1.9303 with an average content of 0.9933. Hg content also showed significant difference between months ($P<0.05$). Difference was seen in February due to concentration below the detectable level.

4.6.2 Sediment

Copper content in the sediment ranged from 0.2973 ppm to 2.9632 ppm with the average content of 0.6521 ppm. Zinc content ranged from 4.8362 ppm to 141.194 ppm with the average content of 33.600 ppm. Cadmium content ranged from 4.426 ppm to 15.0138 ppm with the average content of 7.3384 ppm. Mercury content in the bivalve tissues of the station was found below the detectable level (Fig.21).

4.6.3 Water

Copper ranged from 0.36 mg/l to 3.378 mg/l with an average of 1.329 mg/l. Zinc ranged from 1.0254 mg/l to 2.271 mg/l with an average of 1.4998 mg/l. Cadmium ranged from 0.003 mg/l to 0.018 mg/l with an average of 0.0095 mg/l. Lead ranged from 0.15 mg/l to 0.29 mg/l with an average of 0.2322 mg/l (Fig.22).

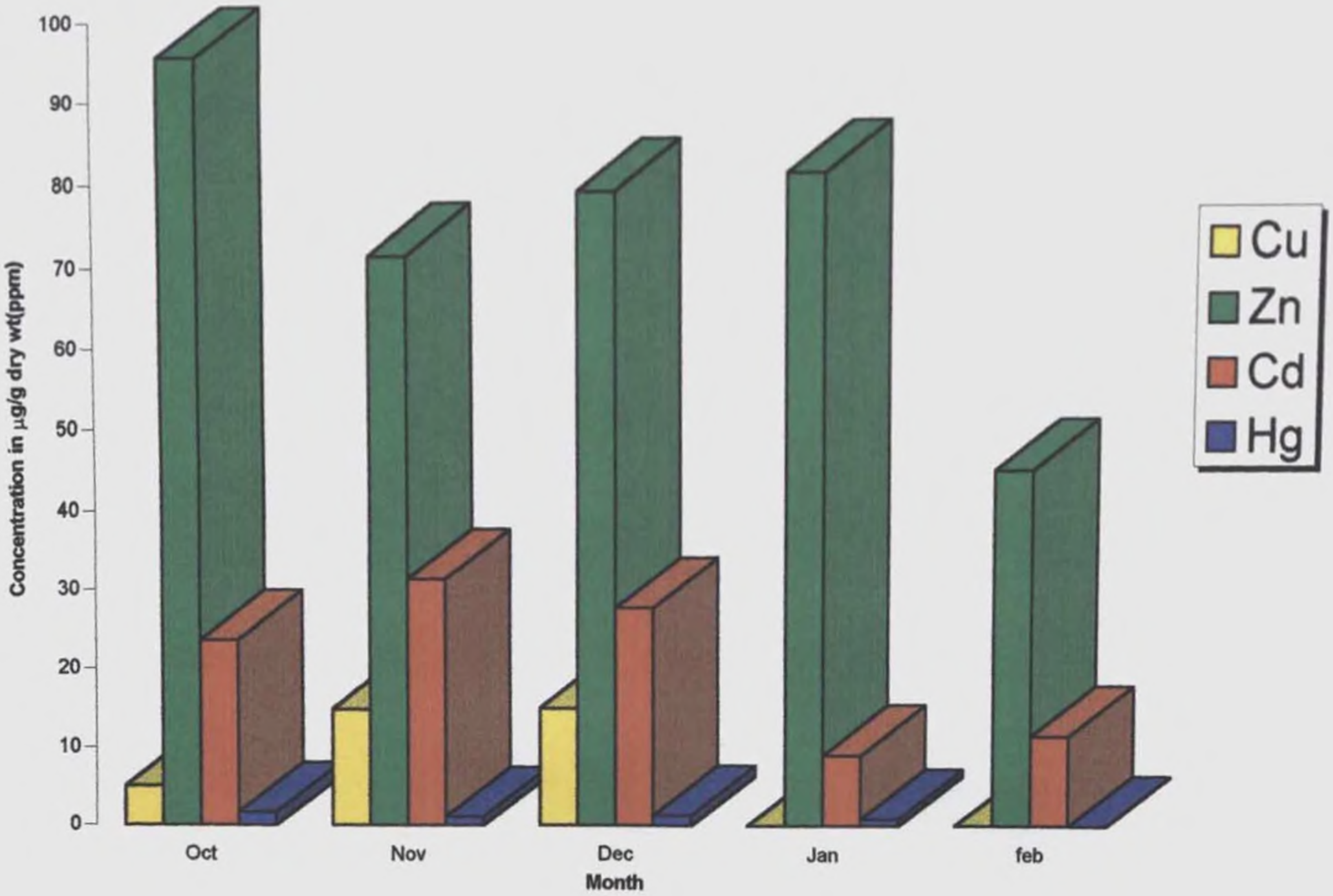


Fig.20 Heavy Metal Content in Bivalves of Station VI

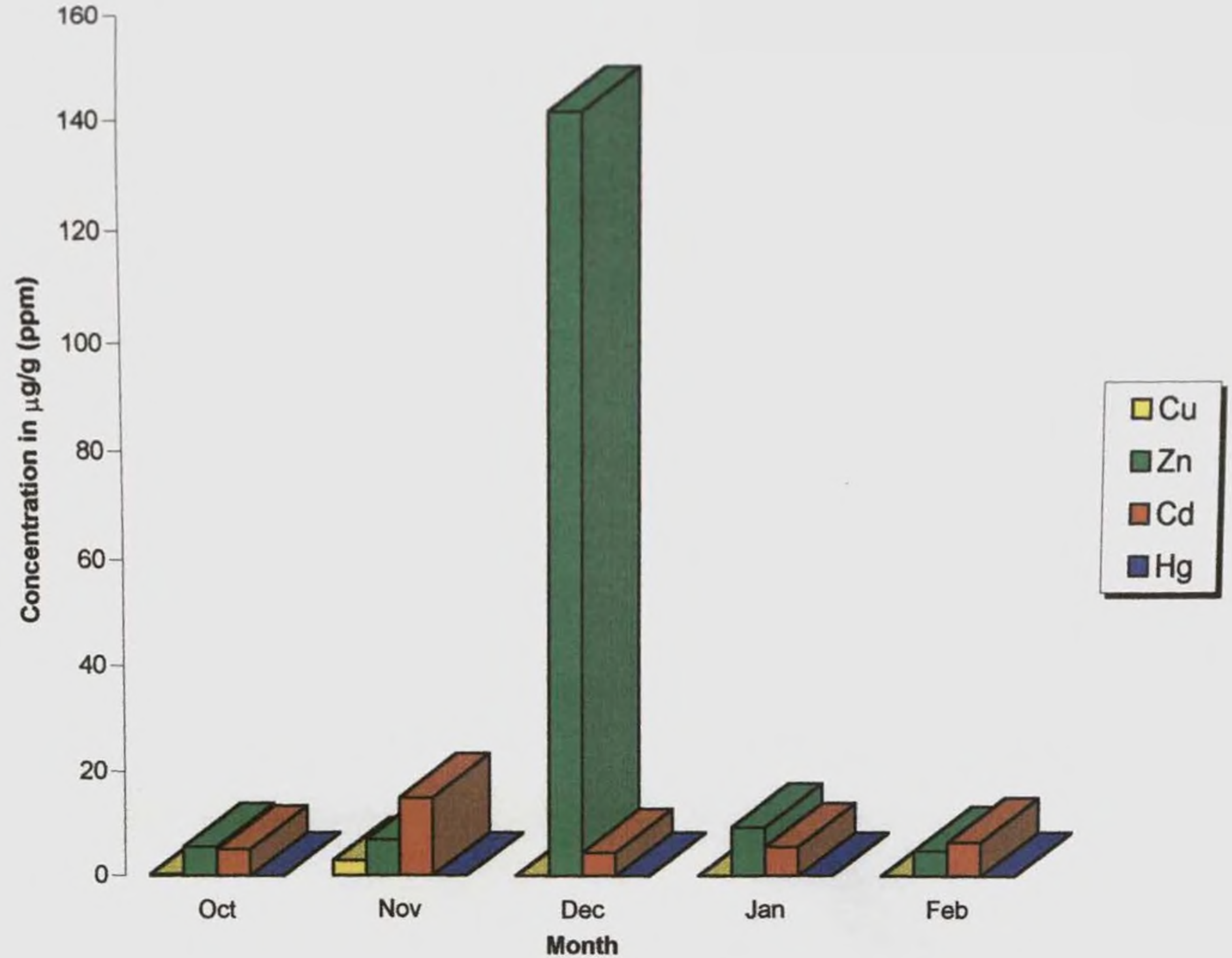


Fig. 21 Heavy Metal Content in Sediment of Station VI

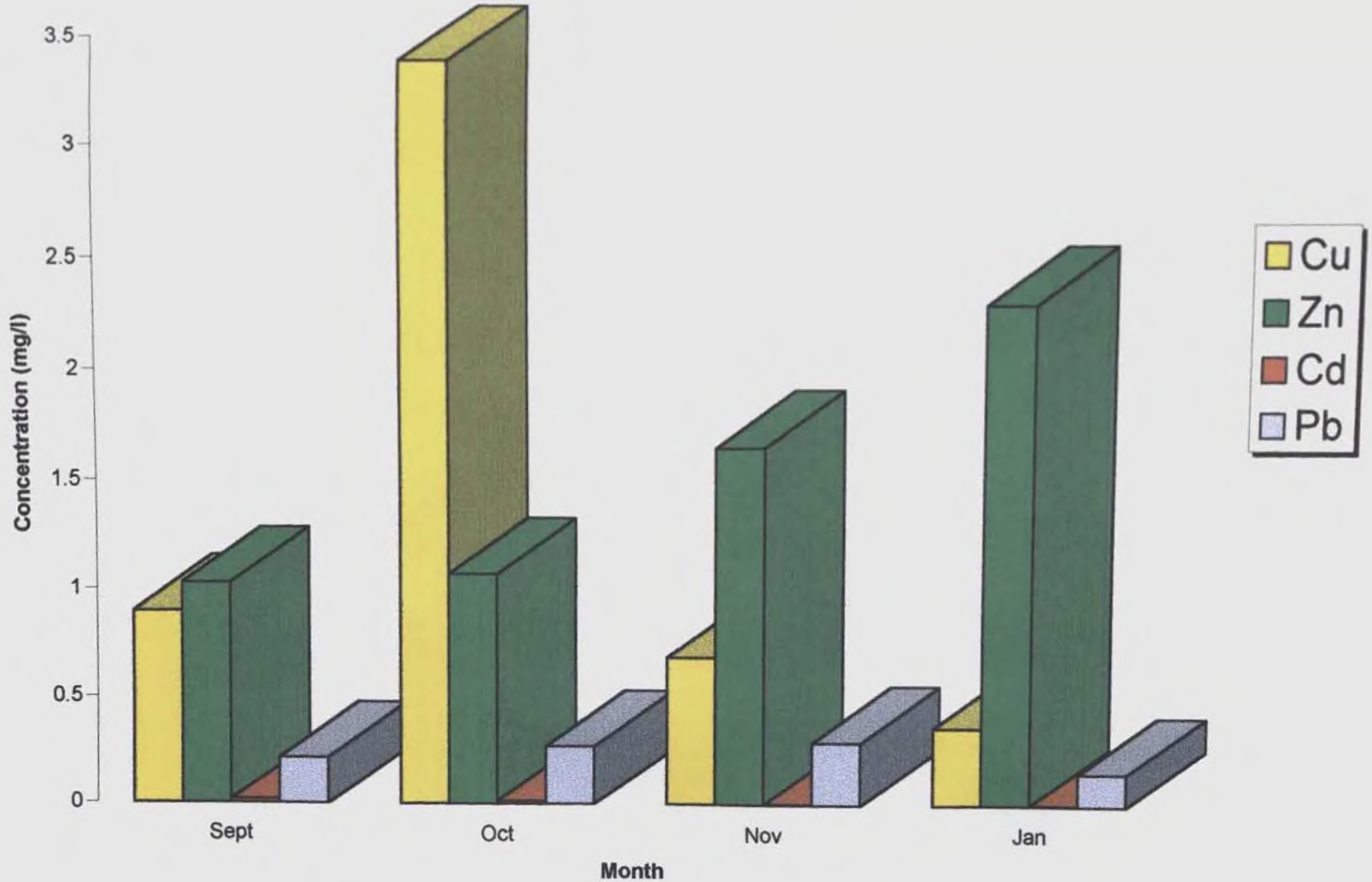


Fig.22 Heavy Metal Content in Water of Station VI

4.6.4 Other Parameters

Salinity ranged from 6.89 ppt to 28.02 ppt with an average value of 21.19 ppt. Temperature ranged from 28^0 C to 29.3^0 C with the average value of 28.74^0 C. Length of the clams varied from 3.5 cm to 4 cm with an average length being 3.7 cm (Fig.23).

4.7. ANOVA and Correlation Studies

Variation of heavy metals in bivalves of different stations was also studied. This comparison study was done between stations I and II and stations III, IV and V, as the species are same among these respective stations. Heavy metals namely Cu, Zn, Cd and Hg did not show significant difference in bivalves of stations I and II ($P > 0.05$ i.e., for Cu, 0.3065;Zn, 0.2072;Cd, 0.6950 and Hg, 0.2928). Between stations III, IV and V, difference in accumulation of Cu was seen in bivalves of III and IV stations ($P < 0.05$). Other metals did not show significant difference between stations. Correlation of heavy metal accumulation in bivalves with parameters like salinity, temperature, dissolved oxygen and length of the bivalve was studied and results are interpreted in tables1-6.

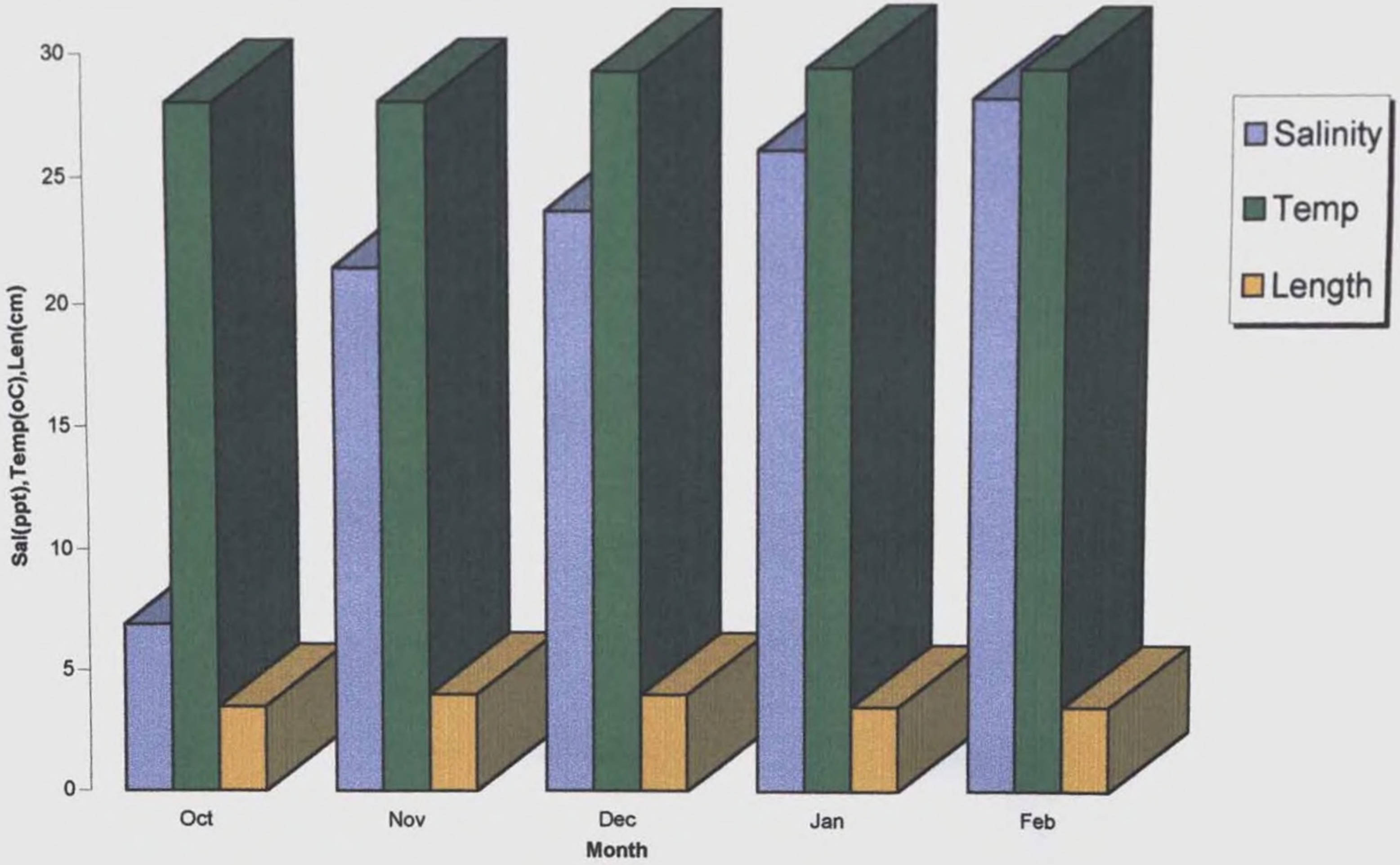


Fig.23 Parameters of Station VI

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	+0.827*	-0.130	-0.359	-0.572
Temperature	+0.273	-0.422	+0.144	+0.116
DO	-0.033	+0.105	0.336	-0.146
Length	+0.344	-0.369	-0.636	+0.046

+ Positive correlation
* Significant correlation
- Negative correlation

Table 1. Correlation of Heavy Metals with other parameters in station I.

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	+0.626	+0.649	+0.040	-0.849*
Temperature	+0.231	+0.213	+0.300	-0.299
DO	+0.277	-0.012	+0.028	-0.471
Length	-0.526	-0.612	-0.681	+0.139

Table 2. Correlation of Heavy Metals with other parameters in station II

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	-0.274	+0.011	-0.133	+0.117
Temperature	+0.349	+0.024	+0.599	-0.223
Length	+0.330	+0.243	-0.118	+0.308

Table 3. Correlation of Heavy Metals with other parameters in station III.

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	+0.392	+0.454	+0.012	-0.262
Temperature	-0.147	-0.098	+0.103	+0.353
DO	-0.24	+0.099	-0.743	+0.440
Length	+0.577	+0.678	-0.583	-0.146

Table 4. Correlation of Heavy Metals with other parameters in station IV.

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	+0.150	+0.219	+0.307	+0.067
Temperature	-0.104	+0.289	+0.664	+0.263
DO	+0.339	+0.028	+0.353	+0.009
Length	-0.703	+0.025	-0.045	+0.528

Table 5. Correlation of Heavy Metals with other parameters in station V.

Metals / Parameters	Cu	Zn	Cd	Hg
Salinity	-0.131	-0.739	-0.427	-0.777
Temperature	-0.387	-0.421	-0.657	-0.580
Length	+0.961	+0.042	+0.819	+0.328

Table 6. Correlation of Heavy Metals with other parameters in station VI.

5. DISCUSSION

The use of aquatic organisms to define areas of trace metal pollution has gained worldwide attention, as these organisms not only concentrate metals from surrounding environment (water and sediment) but also represent the biological availability of metals at each location. Many different species of bivalve molluscs have been used to monitor the concentrations of metals in various aquatic ecosystem(Phillips, 1977 a; Goldberg, 1975; Phillips, 1978; Phillips, 1980; Pillai and Valsala, 1995; Senthilnathan *et al.*, 1998 and Jones *et al.*, 2000). It is reported that indigenous molluscs are so often used as indicators in pollution studies because of their sedentary and comparatively long life, position in the food web and accessibility (Cairns *et al.*, 1971) and sensitivity to copper, zinc and other associated metals, (Cairns *et al.*, 1976 and Guth *et al.*, 1977). Again bivalves are known for their capability of acting as efficient time integrated indicators of various metals over a wide range of environmental conditions (Phillips, 1977 a; Goldberg, 1975; Phillips, 1978; Phillips, 1980; Pillai and Valsala, 1995). There fore in the present study also bivalves are used for the bioaccumulation studies *viz.*, oysters (*Crassostrea madrasensis, Saccostrea cucullata*), clams (*Sunetta scripta*). It is reported that to a large extent the metals in indicator organisms are supplanting that of metals in water or sediments as a means of identifying polluted areas (Phillips, 1979 a). Hence water and sediment samples were also analyzed for presence of heavy metals.

According to Phillips (1977 a), the variables affecting the results of indicator surveys using bivalves remain partly unknown, particularly in terms of their quantitative effects. However, he also stated that, some data are available on the effects of an organism's in relation to age, weight, size, sex, season, position of sampling, salinity, water temperature and the co-existence of several metals on the accumulation or on the final concentrations of metals in bivalves. Therefore in the present work, investigations are conducted on heavy metals in bivalves with various sizes in different seasons in relation to the effects of salinity, water temperature and dissolved oxygen in the aquatic ecosystems.

Salinity differences may strongly alter the bioavailability of pollutants to organisms, and hence may impart toxicity (Mantoura *et al.*, 1978). According to Larsen, (1979) cadmium and zinc varied significantly with salinity. Sundararaj and Krishnamurthy (1972) explained their findings on the non-conservative behaviour of heavy metal increase with decrease in salinity. Present study also shows varying relations of metal accumulation with increasing or decreasing salinity. In the present study it is noted that; mercury showed a significant negative correlation with salinity in station II, where as accumulation of copper in bivalves showed a significant positive correlation with the salinity in station I. Other metals did not show much significant relation with variation in salinity. Wright, (1977) and Vernberg *et al.*, (1977) observed a direct effect of salinity on the rate of uptake of trace metals by marine, estuarine and brackish water organisms. With cadmium in water, reducing salinity also reduces chloride complexation, thereby increasing the bioavailability of cadmium to organisms (Mantoura *et al.*, 1978). Senthilnathan *et al.*, (1998) proved that the metal load in tissues were higher when the medium has lower salt content and statistical analysis of their data confirmed a significant inverse relationship of metal body burdens and salinity.

In the present study the bioaccumulation of heavy metals are found varying with the environmental temperature, but not in the same pattern, which might have been due to the less variation in the temperatures during the period of investigation. This is in accordance with the study conducted by Dougherty (1988) who reported that no significant systematic variations in metal concentrations throughout the course of the study or at different times of year in different bivalves was noticed which may be due to lack of seasonal fluctuation in temperature of Fiji's monotonous maritime climate. Cunningahm (1979) stated that with higher temperature and increased metabolism more rapid turnover of all tissue constituents including metals would be anticipated. Roberts (1978) stated that the toxicity of heavy metals increase with increase in temperature.

Several authors have studied the effects of the age, weight or size of the organism in the accumulation of heavy metals authors and reported that the trace metal concentrations within molluscs are dependent on size (Romeril, 1971; Raymont, 1972; Boyden, 1974; Phillips, 1976 a and 1976 b; Boalch *et al.*, 1981). In the present study copper showed a positive correlation with length of the animal in station I, III and IV and negative correlation in station II and V. While Zinc showed negative correlation in station II, and I cadmium showed negative correlation and mercury showed positive correlation in all the five stations. Mackay *et al.*, (1975) asserted that metal concentrations decrease with increasing age and wet weight of oysters. Raymont (1972) have reported an increase in concentrations of zinc, copper and iron with increased age of *Mercenaria mercenaria*. Boyden (1974) reported relationships between size of organism, metal concentration and total metal content for several species of bivalves. Phillips (1976 a and 1976 b) stated that, in the environment, smaller individuals of *Mytilus edulis* were found to contain significantly higher concentrations of zinc, cadmium, lead and copper than larger individuals. Again Phillips (1977 b) reported that the size or weight dependent variation in concentrations of metals is by no means always the same, as the incidence of significant relationships between tissue size and metal concentration vary with season and between metals. Apart from these it is reported by many authors that in bivalves, Cd concentrations either decrease with growth remaining unchanged, or increase with age (Bryan, 1973; Boyden, 1974; Szefer and Szefer (1985). From the present study also it is understood that the size or weight dependent variation in concentrations of metals is by no means always the same.

Regarding the seasonality and the bioaccumulation of metals the present study revealed that, copper, zinc, cadmium and mercury levels in the animals gave high values in November - December followed by another peak in Monsoon months (Aug). A similar observation was done by Sankarnarayanan *et al.*, (1978) and reported that high concentrations of Zn, Cu, and Fe were observed in *Crassostrea madrasensis* during December to May and low values were confined June to November when the fresh water discharge through rivers were maximum. Fowler and Oregioni (1976), in studies of the variation in the concentrations of ten metals in *Mytilus galloprovincialis*, reported that the seasonal maximum was observed in samples collected in March (spring) which was attributed to the reproductive state of the animals and to the high winter run off increasing the amount of available metals. In a study conducted by Senthilnathan *et al.*, 1998, in south east coast of India, a clear significant seasonal pattern of metal load in the tissues of mussel, *Perna viridis* and oyster *Crassostrea madrasensis* was observed with high level during the monsoon (October-December) period. Similar observations were also reported in *Mytilus edulis* and oyster from Bay of Bourgneuf, France (Amiard *et al.*, 1986) and in three mollusks, *Villorita cyprinoids* var. *Cochinonsis*, *Meritrix casta* and *Perna viridis* in south west coast of India (Laksmanan and Nambisan, 1983). The study conducted by Pattersen *et al.*, (1997) has shown that, high concentration of all metals namely Zn, Mn, Fe & Cu were recorded during the rainy monsoon where as concentrations were low during the dry season in edible marine gastropods. In accordance with these, from the present study it was noted that copper, zinc, cadmium and mercury in general showed high values in monsoon (Aug). However, these concentrations showed the highest peak in November and December. This may be due to the northeast monsoon and the following fresh water run off which might have increased the amount of available metals.

According to Patel and Anthony (1991), on exposure to Cd in the presence of either Zn or Cu or in combination of all three metals [Cd+Zn+Cu], respiratory activity decreased significantly with changes in oxygen consumption. Sze and Lee (2000) asserted that, oxygen consumption is a sensitive physiological response of marine organisms to the presence of heavy metals in the environment. Baby and Menon (1986) and Prabhudeva and Menon (1986) have found the decrease in the oxygen consumption rate in *Perna indica* and *Perna viridis* respectively in the presence of heavy metals. However, an increasing trend has been reported by Krishnakumar *et al.*, (1990) for *Perna viridis* and by Mohan

Raj and Hameed (1991) for the fresh water mussels *Lamellidens marginalis*, on exposure to copper. In the present study, oxygen consumption rate *in-situ* could not be determined, as it is difficult to assess in the natural ecosystem of the organism due to interference of many metals and other environmental parameters. Nevertheless, the dissolved oxygen from the sampling sites was analyzed and correlated with heavy metal content in the bivalves. No significant relation could be found between heavy metals content in bivalves and dissolved oxygen content in waters. Present investigations revealed that, bivalves accumulated heavy metals several times greater than their concentration in water. In an experiment, Nambisan and Lakshmanan (1986) found that, when *Perna viridis* exposed continuously to various trace metals (Hg, Cu, Zn and Pb) they accumulated these metal ions several times greater than their concentration in water. Among the three different types of samples analyzed, higher values of metals were observed in oysters and clams followed by sediment and water which is in accordance with the findings of Rajendran and Kurian (1986). From the present study it is found that Cadmium was above the permissible level (Stickney, 2000 and Anon., 2002) in the bivalves in all the stations during the entire period of study, which was very high compared to the Cd levels in the sediments as well as water. Similarly Mercury contents in the bivalves were also above the permissible limits (Stickney, 2000 and Anon., 2002) in certain months in all the stations, which was again higher than that of the sediments as well as water. Several other authors have also reported that heavy metal accumulation by oysters and other organisms showed species specificity (Zingde *et al.*, 1976; Cuyvers, 1984; Boalch *et al.*, 1981 and Patel and Anthony 1991; Jones *et al.*, 2000). Although there are monthly variations observed in metal concentration evidences of exhibiting any overall definite seasonal pattern in oysters, clams as well as in water and sediment are scarce, as reported by Goldberg *et al.*, (1978), Boalch *et al.*, (1981) and Rajendran and Kurian (1986).

In the present study, heavy metals Cu, Zn, Cd and Hg did not show significant difference in the accumulation pattern in *Saccostrea* *cucullata* from two different stations. While difference in accumulation of Cu was seen in *Crassostrea madrasensis* of stations III and IV, other metals did not exhibit significant difference. This attributed probably to the differences in metal concentrations in the two different rivers and estuaries which could reflect differences in the sources of the metals or variations in the sediment concentration, as asserted by Meyerson *et al.*, 1981. It has been observed in the present study that, oysters of both species accumulate higher concentrations of metals than found in clams. It is apparent that bivalves selected for the present study merit consideration/qualify as bio-indicators in estuarine waters of North Kerala. These are abundant, easily collected and are eaten widely. They appear to have the potential for significant metal accumulation, although further work would need to be done to establish the rate of uptake and elimination and the influence of environmental factors such as salinity and temperature on this process. In the light of the present observations and due to their wide distribution suggests that they could also be considered for monitoring aquatic heavy metal pollution.

SUMMARY

SUMMARY

In recent years, monitoring the concentration of heavy metals in marine and fresh water organisms has received much attention as the people are more conscious about the healthy environment and food. Levels of these metals in a large variety of fish/shellfish would provide baseline data for some of the toxic metals. In the present investigation an attempt has been made to study the presence and distribution of some heavy metals in the bivalve molluscs, *Crassostrea madrasensis, Saccostrea cucullata* and *Sunetta scripta* of the estuarine and mangrove areas of North Kerala. Metal content in the sediments and water samples collected from the same area have also been analyzed to know the accumulation trend. ANOVA was done to asses the monthly variation of metal contents in bivalve of each station and observed significant difference in some months.

In general heavy metals in bivalves showed a maximum accumulation in November - December followed by August – September. The present investigations revealed that, bivalves accumulated most of the heavy metals several times more than that found in water. Accumulation pattern of metals was seen in the order "Bivavles > Sediment > water". The concentrations of metals in the bivalves and sediments were in the order "Zinc > Copper > Cadmium > Mercury > Lead". Among the bivalves, Oysters (*Saccostrea cucullata > Crassostrea madrasensis*) accumulated high concentration of metals followed by clams *Sunetta scripta*.

Biomagnification of zinc was found highest in the months of November – December in all the stations in the bivalves. The oyster, *Saccostrea cucullata* accumulated highest concentrations of zinc. Zinc contents in the sediments of these

stations also showed a similar trend of maximum accumulation in November- December. However, comparatively low level of zinc was found in the sediments in all other months. Zinc content in the water was very less in all the stations though out the period of study. Copper was the next highest metal accumulated in the bivalves and sediments during the course of study in all the stations and in all months. Here also the maximum accumulations were in November -- December months in bivalves as well as in sediments. Among the metals studied Copper was the dominant metal found in the water samples taken from all the stations during the period of investigation. The highest concentration of copper was in September. Cadmium was above the permissible level in the bivalves in all the stations during the entire period of study. Sediments accumulated less Cd compared to bivalves in all the stations in all the months. Cadmium content in the water was very less in all the stations during the entire study period. Mercury contents were above the permissible limits in bivalves during certain months (mainly August-September) in all the stations. The pattern of accumulation in different bivalves did not exhibit much difference. Mercury content in the sediments was found very less in all the stations in all the months except in one station; that showed high values in August - September which is attributed to heavy run off during south west monsoon.

Correlation studies carried out for the metal concentrations in bivalves and parameters like salinity, temperature, dissolved oxygen of the environment and length of the animal did not show significant results. Temperature throughout period of study was found less fluctuating in all stations. To put it in a nutshell the present work portrays the state of art of heavy metals in selected edible bivalves in relation to their environment in the estuaries and mangroves of north Kerala.

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